Anisotropic conduction and ventricular tachycardia

ANISOTROPIC CONDUCTION AND VENTRICULAR TACHYCARDIA

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CHAPTER I

INTRODUCTION

1.1. ANISOTROPIC IMPULSE CONDUCTION

Deviations from the normal sinus rhythm of the heart may have serious pathophysiological implications. The consequences range from only minor impaired pump function during extrasystoles, to complete pump failure during ventricular fibrillation, causing sudden death.

Essentially the disturbances in heart rhythm can be divided into two different groups (Hoffman and Rosen, 1981). 1. Arrhythmias caused by abnormalities in impulse formation and 2. Arrhythmias based on disturbances in impulse conduction. 3. Arrhythmias caused by a combination of abnormalities in impulse formation and disturbances in impulse conduction. Although it is often difficult to distinguish between these mechanisms and disturbances in impulse formation may also cause serious arrhythmias, in this thesis we will only discuss the mechanisms underlying disturbances in impulse conduction.

The propagation of electrical activity first described and demonstrated by Engelmann (1874, 1875) has now been subject of study for more than a century. After the first estimations of conduction velocity by Engelmann in 1875, Burdon-Sanderson and Page (1879) established the criteria for measuring the velocity of electrical propagation in cardiac muscle. According to them conduction velocity had to be measured by calculating the difference between the arrival times at two points, positioned in line with a homogeneously propagating wavefront. These criteria still hold true and it is possible that other ways of indirect measurement of the conduction velocity may yield large deviations from the actual value (Spach et al., 1981, Spach, 1983, Spach and Kootsey, 1983) as will be discussed later.

Detailed studies of impulse conduction became within reach after the introduction of the string galvanometer by Einthoven in 1901. Lewis and Rothschild (1915) came up with the actual rates of propagation in Purkinje and ventricular muscle and in a later

paper a relation between the different types of cardiac fibers and conduction velocity was proposed by Lewis (1924). By that time the effects of tissue anisotropy were thought to be of minor importance although Lewis and Rothschild (1915) noted already some differences between conduction velocity parallel and perpendicular to the fiber direction in the right ventricle.

After the early studies of Lewis and Rothschild it was only thirty years later that the effects of fiber direction on conduction velocity were described by Dräper and Mya-Tu (1959) and Sano et al. (1959). A conduction velocity of 60 cm/s was reported during impulse conduction parallel to the fiber axis opposed to a conduction velocity of only 20 cm/s during conduction perpendicular to the fiber orientation. These values were recently confirmed by studies of Clerc (1976) and Spach et al. (1981, 1982b). Spach also was the first to suggest that anisotropy may play an important role in the initiation of reentrant arrhythmias (Spach et al., 1981, 1987). They stated that conduction disturbances, long thought to be due to "depressed segments" (Schmitt and Erlanger, 1928) or local differences in excitability (Mines, 1914, Hoffman and Cranefield, 1960, Han and Moe, 1964, Cranefield et al., 1971, Cranefield and Hoffman, 1971, Allessie et al, 1976, Kuo et al., 1983, Kimura et al., 1986) may also be caused by geometrical factors influencing the safety factor for conduction in anisotropic myocardium (Spach, 1981, 1982a, 1986, 1987).

Recent studies on the mechanisms of ventricular tachycardia by Wit et al. (1987) and Dillon et al. (1988), demonstrated that tissue anisotropy played an important role in the occurrence of conduction block and reentry in ventricular myocardium surviving myocardial infarction.

1.2. STRUCTURE OF VENTRICULAR MYOCARDIUM.

Ventricular cells have an average diameter of 10 to 20 μ and a length ranging from 60 to 100 μ (Sommer and Dolber, 1979). The cells are more or less cylindrical in shape. The surface however is curved and sculptured into several step like terraces (Sommer and Dolber, 1979). As a result cells can be coupled to several adjacent cells. Ventricular cells are closely coupled to each other by nexuses (Sjöstrand and Anderson, 1954, Dewey and Barr, 1962, De Mello, 1982). The nexuses allow current flow from cell to

cell and are essential for normal impulse conduction (Barr et al., 1965, Weidmann, 1966, 1970, De Mello et al., 1969, Daniel et al., 1976).

At the nexus the cells are in intimate contact, the average width of the gap being 200 Å. In its central area several structures are present bridging the gap with a periodicity of 100 Å (Loewenstein,1981, De Mello 1982). The nexus is composed of subunits, the connexons, which create direct pathways between adjacent cells. Each connexon is separated from the extracellular space by a lipid wall of high resistivity (Loewenstein, 1981). The nexus is a rather unstable structure and may undergo morphological changes related to a low or high resistance state (Dewey and Barr, 1962, Peracchia, 1973, De Mello, 1982).

Calcium appears to play a key role in the regulation of the opening and closure of the nexus, which reaction is probably mediated by calmodulin (De Mello et al., 1969, De Mello, 1982, Sheung, 1980). If the calcium concentration at the nexus rises the channels close and the resistance increases. This is an important mechanism to protect cells against short-circuiting when one of the surrounding cells loses its integrity either mechanically or during hypoxia (Deleze, 1965, De Mello et al., 1969, 1972). After injury first the resistance decreases and the space constant for electrical current increases, but after a short time period the space constant decreases below its normal value and the damaged cell becomes "disconnected" (Wojtczak, 1979, De Mello, 1982). This process of "healing over" was already described by Engelmann (1877) who noted that if one end of a fiber was damaged, impulse conduction was impaired first but after a short time period conduction recovered.

The nexuses form almost always end-to-end connections between the cells, although sometimes side-to-side connections are found (Sommer, 1983). The number of nexuses is directly related to the dimension of the cells, large cells having more nexuses than smaller cells (Jewett, 1971).

The cells are organized into bundles consisting of 2 or more cells. These "unit" bundles (Johnson and Sommer, 1967) are separated from each other by extracellular space. Within each bundle the cells are well connected to each other by multiple nexuses. Adjacent bundles however may be connected by only one nexus. Sometimes a complete fusion of two bundles may occur forming a new and larger bundle. This bundle may fuse or divide into two or more new bundles. At a somewhat larger scale adjacent bundles may be divided from each other by blood vessels or collageneous tissue. During aging bundles become more and more separated because of the inter-

position of collageneous septa. It is apparent that such discontinuities may induce barriers for impulse conduction (Spach et al., 1981, 1982a, 1986).

Both left and right ventricle are three-dimensional structures with an unique and complex geometry. Muscle fibers in the ventricular wall are orientated in helical pathways depending on their position between epicardium and endocardium. The ventricular wall can be divided in about ten or eleven different layers, in each layer the myocardial fibers are organized more or less parallel to each other. The helix angle changes gradually from epicardium to endocardium from about +60 to -60 degrees, like in a Japanese fan (Streeter, 1979).

1.3. CONDUCTION OF THE CARDIAC IMPULSE IN ANISOTROPIC MYOCARDIUM.

Although substantial differences exist between nerve and cardiac muscle, myocardial impulse conduction has long been treated as if occurring in a one dimensional continuous cable (Weidmann, 1952, Noble, 1962, 1966 , Fozzard, 1979, Walton and Fozzard, 1983a, 1983b . The electrical constants for impulse transmission along an electrical cable were determined by Kelvin (1855). The first attempts to apply the cable equations to cardiac myocardium were performed by Weidman (1952). After the numerical solutions of the cable equations by Hodgkin and Huxley (1952) for impulse conduction in the squid axon, Weidmann (1952) applied the cable equations to sheep Purkinje fibers. Although Purkinje fibers consisted of multiple cells generally the experimental results fitted well with the values predicted by the cable equation (Fozzard, 1979).

The cable equation for constant conduction velocity is (Hodgkin and Rushton, 1946, Walton and Fozzard, 1983):

$$\frac{a}{2R_i\theta^2} \cdot \frac{d^2V}{dt^2} = C_m \cdot \frac{dV}{dt} + I_i$$

Where a is the radius of the fiber, R_i is the specific resistivity of the intracellular fluid, θ is the conduction velocity, C_m is the membrane capacitance per unit length, V

is the membrane potential per unit area, I_i respresents the sum of the instantaneous ionic currents per unit area of membrane, and t represents time.

From the cable equation a linear relationship between V_{max} (dV/dt_{max}) and the square of the conduction velocity can be derived (Hodgkin, 1954). Because of this, it has become common practice to estimate the conduction velocity by measuring the V_{max} (Cohen et al., 1984, Buchanan et al, 1985). The upstroke velocity of the action potential is determined mainly by the magnitude of the fast sodium inward current.

In general this seems to fit well with the experimental results and thus was thought to provide information about the relation between the fast sodium inward current and the conduction velocity under a variety of circumstances (Cohen and Strichartz, 1977, Cohen et al., 1984, Buchanan et al., 1985).

The validity of the simple and direct relationship between V_{max} and conduction velocity was challenged by a number of observations (Dominquez and Fozzard, 1970, Spear and Moore, 1974a, 1974b, Saito et al., 1978). Dominquez and Fozzard (1970) showed that when the potassium concentration was increased the conduction velocity showed a biphasical pattern. Despite a continuous decrease of V_{max} the conduction velocity first increased when the potassium concentration was increased to about 7.5 mM and then slowed down when the concentration was further increased. This was explained by assuming, that when the resting membrane potential shifted to less negative values, depolarization of the membrane to threshold voltage required less excitatory current (Domiquez and Fozzard, 1970). At higher potassium concentrations conduction velocity became more and more depressed due to the depression of the Vmax.

According to the continuous cable theory the shape and the time course of the action potential can be altered only by altering the membrane properties of the cells. Alterations of the axial resistance should only modify conduction velocity but not the shape and time course of the action potential (Spach et al., 1981).

For a constant conduction velocity and constant membrane properties Hodgkin (1954)

$$\text{derived } \theta^2 = k \cdot \frac{1}{R_i}$$

where k is a constant depending on the membrane properties. Hodgkin concluded that R_i and θ can be changed reciprocally without altering the time course of the action potential. When the axial resistance increases, conduction velocity decreases proportionally.

Although Dräper and Mya-Tu (1959) and Sano et al. (1959) already demonstrated that conduction velocity changes by changing the direction of impulse propagation it was not until recently that attempts have been made to quantify the effects of tissue anisotropy on impulse conduction. Because of the anisotropy of the ventricular myocardium the axial resistance is different along and perpendicular to the fiber axis. Sperelakis and MacDonald (1974) measured the ratio between longitudinal and transverse resistivities and came up with a ratio of 11.3. Experimental evidence that the anisotropic distribution of the conduction velocity is caused by differences in axial resistance was also given by Clerc (1976). Trabecular bundles from the right ventricle of the calf heart were used and studied at a low temperature of 25 ^{o}C (to prevent contracture of the muscle). The ratio between longitudinal and transverse intracellular resistance was 9.4. Because conduction velocity is related to the square of the axial resistance this resulted in a ratio of 3.0 between longitudinal and transverse conduction velocity. Action potential configuration was not influenced by the direction of impulse spread. Clerc (1976):" For widely different external and internal resistances, thus different conduction velocities, the shape of the propagating action potential is the same, as would occur if it was determined exclusively by the properties of the same surface membrane" and from this it emerged that the cable equations fitted well with experimental results.

Spach et al. (1981, 1982a, 1987) however did note consiberable effects on configuration and time course of the action potential when the direction of impulse spread was changed. This led him to conclude that the cable equation did <u>not</u> adequately describe impulse conduction in anisotropic myocardium. In a one dimensional continuous cable a high conduction velocity is related to a fast rate of rise of the action potential (V_{max}) and a low τ-foot (which represents the time course of the membrane potential during the foot of the action potential). In anisotropic myocardium, during slow conduction perpendicular to the fiber axis V_{max} was significantly higher than during fast conduction parallel to the fiber axis. Also τ-foot was shorter during slow transverse conduction and lengthened when the conduction velocity increased (Spach et al.,

1981, 1987). These deviations from the cable theory were explained by Spach by taking into account the effects of recurrent axial resistances in the form of nexuses. Such barriers separate the active membranes in loosely coupled patches and will reduce the space constant of electrical current spread. Because of the elongated cell shape and the packing in parallel bundles relative few barriers are present in a longitudinal direction (Johnson and Sommer, 1967, Sommer and Dolber, 1979). In a longitudinal direction the space constant for electrotonic current thus is higher and can flow over a considerable distance (Spach et al., 1981). As a result of this high electrotonic current flow in a longitudinal direction, V_{max} decreases despite a high conduction velocity. The increase of the space constant also increases the τ-foot of the action potential (Spach et al., 1981). In a transverse direction however electrotonic current spread is less and more current is confined within the cells because of the insulating high resistance barriers. Thus V_{max} is higher and τ-foot shorter at a lower conduction velocity (Spach et al, 1981, Delmar et al., 1987).

When the axial resistance increases to very high levels the cells become more and more separated and Vmax further increases until a value is reached which can be found under space clamp conditions. This new concept of discontinuity led to a formerly unknown type of cardiac impulse conduction during which the impulse "jumps" from cell to cell and the delay between the cells increases. Conduction velocity in normal myocardium thus may decrease to values of less than 5 cm/s, previously only measured in depressed myocardial segments (Cranefield et al., 1971). The occurrence of notches during and after the depolarization phase of the action potentials indeed suggests that conduction is discontinuous. The appearance of notches is caused by the depolarization of the next cell after a delay during which the impulse passes the nexus (Spach et al.,1981, 1987).

Simulation studies by Joyner (1982) using the model of coupled membranes (Beeler and Reuter, 1977) and Diaz et al. (1983) using a core conductor model demonstrated that when the effects of the nexus resistances were incorparated by including a periodic resistance, impulse propagation became discontinuous which was reflected by the occurrence of notches in the action potentials and multiple low amplitude spikes in computed extracellular potentials.

Spach et al. (1981) further postulated that the geometrical factors in anisotropic myocardium, also affect the "safety factor" for conduction. The "safety factor" is a rather poorly defined parameter (Spach et al., 1981, Schmitt and Schmitt , 1940) and reflects

the ratio between the maximal current a cell can supply and the current necessary to bring the surrounding cells to threshold. Because of the large current load and the resulting low V_{max} longitudinal to the fiber axis the safety factor during fast longitudinal conduction is supposed to be lower than during slow transverse conduction (Spach et al., 1981). As a result, a lowering of the stimulating efficacy of the depolarisation wave by depression of the fast sodium inward current should cause conduction to fail first in a longitudinal direction. Spach et al. (1981) came up with some experimental evidence showing that when the take-off potential was lowered by premature beats, longitudinal conduction became decremental or blocked while slow transverse conduction still proceeded. These findings were confirmed by Tsuboi et al. (1985) who demonstrated that when the safety factor was lowered by high concentrations of extracellular potassium, the V_{max} of the action potential measured during longitudinal conduction was depressed to a greater extent than during transverse conduction.

Computer simulation studies

The computer simulation studies of Van Capelle (1983) and Rudy et al. (1987) however challenged this concept of preferential longitudinal conduction block. Although they confirmed that over a small range of disc resistances, V_{max} decreases when the coupling resistance decreases, they showed that at very low coupling resistances the cable loses its lumped character and Vmax tends towards a stable value (Van Capelle, 1983). Furthermore when the disc resistances were increased to high values (Rudy et al., 1987) a rapid decline of the V_{max} was reported. This decline of V_{max} is probably caused by the occurrence of slow foot potentials which inactivated the sodium inward current (Van Capelle, 1983, Rudy et al., 1987).

They also found a different relationship between the τ-foot of the action potential and the axial resistance. When the disc resistance increased, τ-foot also increased, instead of decreasing as reported by Spach et al. (1981). Under all different circumstances, conduction block always occurred more promptly during slow transverse conduction than in a longitudinal direction. Recent experimental studies by Delmar et al. (1987) demonstrated that when the nexus resistance was increased by heptanol, regardless of the changes in V_{max}, transverse conduction block always occurred before longitudinal conduction block. This conduction failure was caused by the occurrence of slow foot potentials partially inactivating the fast sodium inward current. In a

recent paper Spach et al. (1987) postulated that the opposite results concerning the relationship between the τ-foot of the action potential and the axial resistance can be explained by assuming that the membrane capacitance is different depending on the direction of impulse spread. However there is still no consensus on the role of anisotropy in the occurrence of local conduction block.

Extracellular potentials

Fiber geometry and morphology also has notable effects on the recorded extracellular potentials. In a one dimensional continuous cable the magnitude of the extracellular potential is primarily determined by the intracellular potential gradient (Rall, 1969, Spach et al., 1972, 1973, Spach and Barr, 1975, Barr, 1984). However it was shown by Spach et al. (1979, 1986) and Rudy et al. (1987) that in anisotropic myocardium the extracellular potentials are determined by the axial current flow. Because of the large axial current flow in a longitudinal direction the amplitude of the extracellular potentials is larger than during transverse conduction. Both experimental work and simulation studies showed that the extracellular potential of the wavefront indeed rapidly declines when the direction of impulse spread changes from longitudinal to transverse conduction despite an increase of the action potential amplitude (Roberts et al. 1979, Spach et al. 1979). During fast longitudinal conduction a large positive R wave preceeds the high amplitude intrinsic deflection, while during transverse conduction a low amplitude negative intrinsic deflection preceded by a small or even absent R wave is recorded. When during aging or after myocardial infarction the degree of anisotropy increases by progressive separation of adjacent myocardial bundles by collageneous septa (Spach et al., 1986, Spear et al, 1983a, 1983b, Richards et al., 1984) longitudinal conduction is not affected and the shape of the extracellular deflections is not changed. In a transverse direction however the impulse now is forced in a complex zig-zag pathway which is reflected in the extracellular electrogram by multiple low amplitude deflections, reflecting the activation of separate but adjacent myocardial bundles near the recording site.

1.4. REENTRANT EXCITATION

The concept of reentrant excitation as a mechanism of ventricular fibrillation was introduced by MacWilliam in 1887. Mayer (1906, 1908), Mines (1913, 1914) and Garrey (1914) showed that reentrant excitation could be initiated in ring preparations cut from the jelly fish or from the hearts of tortoise, dogfish, dog and cats. The conditions necessary for initiation of reentry were recognized by Mines (1913, 1914): 1. Block of an impulse at some site within the myocardium. 2. Slow conduction over an alternate pathway, 3. Delayed activation of tissue beyond the block and 4. Reexcitation of tissue proximal to the block.

Two different types of reentrant excitation can be distinguished: 1. Circus movement excitation involving an anatomical barrier (Mines, 1913, 1914, Lewis et al., 1920, 1921, Rosenblueth and Garcia Ramos, 1947, Page et al., 1983, Frame et al., 1986) and 2. Circus movement excitation involving a functionally determined area of conduction block (Moe, 1962, Allessie et al., 1977, Wit et al., 1987, Dillon et al., 1988)

Reentrant excitation involving an anatomical barrier.

Lewis et al. (1920) demonstrated the presence of reentrant excitation in atrial flutter in the dog heart by mapping the spread of excitation during the arrhythmia. They showed that the impulse circulated around the orifices of the superior and inferior caval veins. Rosenblueth and Garcia Ramos (1947), Kimura et al. (1954), Hayden et al (1967), Page et al. (1983), Frame et al. (1986) further studied the principles of reentrant excitation involving an anatomical barrier. When the impulse circulates around an anatomical obstacle the length of the circular pathway is determined by the size of the anatomical barrier. Usually the length of the excitation wave (e.g. the product of conduction velocity and refractory period) is shorter than the length of the reentrant pathway and consequently an excitable gap between the crest and the tail of the circulating impulse is present. The cycle length of the arrhythmia is determined by the length of the circular pathway and the conduction velocity (Lewis, 1925). Because the length of the circuit is fixed, the rate of the arrhythmia can be modified only by changing the conduction velocity. Due to the presence of an excitable gap, it is possible to entrain, reset or terminate these tachycardias by programmed electrical stimulation (Wellens et al., 1975b, 1978, Waldo et al.,1987, Frame et al., 1986).

In man reentrant excitation around an anatomical obstacle was first demonstrated in patients suffering from the Wolff-Parkinson-White syndrome (Durrer et al., 1967a, 1967b, Durrer and Wellens, 1974, Wellens, 1975b, 1975c, Wellens et al., 1980, 1984). In these patients the impulse propagated in a circuitous pathway involving the atria, ventricles, A-V node and an accessory pathway (Kent, 1913). This reentrant arhythmia, already predicted by Mines (1914) can be terminated by cutting the accessory bundle (which is the ultimate proof that the arrhythmia is caused by reentrant excitation, Mines, 1913). Although direct evidence is not yet available it is likely, that also sustained ventricular tachycardia initiated in the chronic phase after a myocardial infarction is based on reentry around an anatomical barrier (Wellens et al., 1974, Josephson and Wellens, 1984, De Bakker et al., 1983).

Reentry without the involvement of gross anatomical obstacles

Although Garrey in 1914 already postulated that anatomical barriers are not essential for the maintaince of reentrant excitation, it was not until sixty years later, that Allessie et al. in 1973 demonstrated circus movement excitation in atrial myocardium without the involvement of a gross anatomical barrier. The characteristics of functional reentry, as postulated in the leading circle theory are (Allessie et al., 1977): 1. Absence of an anatomical obstacle in the centre of the circuit. 2. No excitable gap between the crest and tail of the circulating impulse. 3. The length of the circuit is determined by the wavelength of the impulse (i.e. product of refractory period and conduction velocity). 4. The cycle length of the reentrant tachyarrhythmia is determined by the duration of the refractory period. 5. Size and localization of the circuit are not fixed, but may vary by changes in electrophysiological properties. Leading circle reentry is considered to be an important pathophysiological mechanism of atrial or ventricular fibrillation. Moe and co-workers (1962, 1964) postulated that due to local differences in refractoriness, multiple areas of conduction block developed resulting in degeneration of the impulse into multiple wavelets. These chaotically wandering wavelets are fully determined by the functional properties of the myocardium. Recently Allessie et al. (1985) provided experimental evidence for the multiple wavelet theory.

In conclusion a number of important differences exists between anatomically and functionally determined reentry circuits.

1. The reentrant pathway is determined by the dimension of the anatomical barrier during "anatomical" reentry. In the leading circle type of reentrant excitation the dimension of the circuit are determined by the electrophysiological properties of the myocardium and the length of the circuit can change with alterations in electrophysiologic properties.
2. During "anatomical" reentry usually the length of the reentrant pathway exceeds the length of the excitation wave and consequently an excitable gap is present. Because during functional reentry the length of the circuit is equal to the length of the excitation wave no excitable gap is present.
3. Due to the presence of an excitable gap anatomical circuits are stable and long lasting, while functional reentry tends to terminate spontaneously.
4. The cycle length of anatomically determined circuits is inversely related to the conduction velocity wheras in leading circle reentry the revolution time is proportional to the refractory period.

1.5. REENTRANT EXCITATION AS A MECHANISM OF SUSTAINED VENTRICULAR TACHYCARDIA

Ventricular tachycardia is an arrhythmia arising distal to the subdivision of the bundle of His, caused either by the rapid discharge of an ectopic focus or by reentrant excitation within the ventricles. Not only the high rate of ventricular tachycardia may lead to impaired pump function of the heart, but also degeneration into ventricular fibrillation frequently occurs resulting in sudden cardiac death (Moss et al., 1977, Moss 1980). Ventricular tachycardia and ventricular fibrillation occur most frequently in patients suffering from coronary heart disease. During the acute phase of myocardial infarction ectopic ventricular beats and short periods of ventricular tachycardia frequently occur (Lie et al., 1975). The mortality rate during the acute phase of myocardial infarction is about 20 to 30 %. After this phase, which lasts for about 30 minutes,

sinus rhythm returns and less arrhythmias occur. However after about five hours, a second period of arrhythmias starts which may last for several days. In the chronic phase of myocardial infarction in some patients episodes of ventricular tachycardia or ventricular fibrillation occur. In this phase, both nonsustained or sustained ventricular tachycardia can be initiated by programmed electrical stimulation (Wellens et al., 1974). Both clinical (Wellens et al., 1974, 1975a, Josephson et al.,1978, 1979, 1982, 1984, Josephson, 1986, De Bakker et al., 1983) and experimental studies (El-Sherif et al., 1977b, 1981, 1985, Mehra et al., 1983, Gough et al., 1985, Wit et al., 1982, 1987, Kramer et al., 1985, Garan et al., 1987, Pogwidz and Corr, 1987, Dillon et al., 1988) have demonstrated that reentry is the most likely mechanism of sustained ventricular tachycardia after myocardial infarction. High resolution mapping studies revealed that reentrant circuits within surviving myocardial layers overlying the infarct may be the source of some ventricular tachycardias (El-Sherif et al., 1981, 1985, Mehra et al., 1983, Cardinal et al., 1984, Wit et al., 1982, 1987 and Dillon et al., 1988). The border zone myocardium of the infarct consists of thin layers of surviving myocardium. Between these fibers destroyed and necrotic myocardial cells are found (Spear et al., 1983a, 1983b, Richards et al., 1984, Gardner et al., 1985). These surviving myocardial cells have normal electrophysiological properties (Spear et al. 1983a, 1983b, Richards et al., 1984, Gardner et al., 1985). However due to the interposition of collageneous fibers and necrotic myocardial cells conduction velocity during transverse impulse propagation decreases significantly (Spear et al., 1983a, 1983b). The reduction of the conduction velocity is accompanied by the appearance of fractionated deflections in the extracellular electrograms and a reduction of the space constant of electrical current flow in a transverse direction (Spear et al., 1983a, 1983b, Gardner et al., 1985). Because the longitudinal conduction velocity is not altered the ratio between longitudinal and transverse conduction velocity increases. The studies of Wit et al., (1987) and Dillon et al. (1988) have provided evidence that conduction block and reentry could occur due to this enhanced degree of anisotropy. They demonstrated that the reentrant loop was circulating around a line of functional conduction block, orientated parallel to the epicardial fiber direction.

Anisotropy may also play an important role in ventricular tachycardia in patients. Epicardial and endocardial mapping studies during cardiac surgery revealed the presence of "continuous" local activity near the site of "earliest" activation during ventricular tachycardia. These fragmentated electrograms may reflect areas of poor elec-

trical coupling and non-uniform anisotropic conduction. However it is still uncertain whether these areas are part of small reentrant circuits (Josephson et al., 1978, 1982, 1984, Fenoglio et al., 1983). In a recent study Brugada et al.(1985) demonstrated that, although in about 40% of the patients the anatomical substrate causing continuous activity is present, only a minority of the patients will actually suffer from periods of sustained ventricular tachycardia.

1.6. AIM OF THE STUDY

Although a number of theoretical problems of anisotropic conduction have been resolved there is still considerable debate about the role of anisotropy in the genesis of conduction block and reentry (Spach et al., 1981, 1982, 1986, 1987, Van Capelle, 1983, Rudy et al., 1987, Wit et al., 1987, Dillon et al., 1988). One of the objectives of this study was to investigate the role of tissue anisotropy in impulse conduction and conduction block. In the left ventricle of a rabbit heart, perfused according to the Langendorff technique, conduction velocity and refractory period were measured and the wavelength of refractoriness (product of conduction velocity and refractory period) was calculated. Because of the anisotropic morphology the conduction velocity and the wavelength were measured in two directions, e.g. parallel and perpendicular to the fiber orientation. Conduction parallel to the epicardial fiber direction was about three times faster than conduction perpendicular to the fiber direction, i.e. the ratio between longitudinal and transverse wavelength was about three. Although slow transverse conduction and the short transverse wavelength should facilitate the initiation of reentry (Smeets et al., 1986, Rensma, 1987, Rensma et al., 1988) no ventricular tachycardias could be induced in the intact rabbit ventricle. Due to the three dimensional impulse spread, slow transverse impulse conduction did not occur over a long distance, because within a short distance it was bypassed by fast conduction along an alternative route. This three dimensional spread of activation complicated the detailed study of anisotropic conduction and conduction block. To facilitate the study of anisotropy we developed an experimental two-dimensional model of anisotropy. By destroying the intramural and endocardial layers of the ventricle by liquid nitrogen it was possible to create a perfused epicardial layer. The electrophysiological properties (conduction velocity, refractory period and maximal pacing rate) of this two-dimensional epicardial layer were not significantly different from those measured in the in-

tact ventricle. However due to the removal of the intramural and endocardial layers, slow transverse impulse conduction was no longer interrupted by epicardial breakthrough and the effectivity of anisotropy was greatly enhanced.

Another aim of this study was to evaluate the role of anisotropy in the initiation of reentry. Wit et al. (1987) and Dillon et al. (1988) demonstrated that local loss of myocardial tissue in the healing phase after a myocardial infarction enhanced the degree of anisotropy and may facilitate the initiation of sustained ventricular arrhythmias. However in these studies ischemic alterations of the cells also might have contributed to the enhanced risk of the initiation of reentry. In the experimental epicardial layer created by freezing it was possible to study the role of tissue anisotropy in the initiation of reentrant ventricular tachycardia without ischemic alterations of the cells. After freezing sustained ventricular tachycardia could be easily initiated by electrical stimulation. High resolution extracellular mapping (simultaneous recording of electrical activity at 192 different epicardial sites) was performed to reconstruct the spread of excitation during tachycardia. Reentrant excitation around a functional line of conduction block turned out to be the underlying mechanism of these tachycardias. However in contrast with the leading circle type of reentry (Allessie et al., 1977) these tachycardias possessed a clear excitable gap. Due to the presence of an excitable gap the tachycardias were stable and longlasting.

CHAPTER II

ANISOTROPIC CONDUCTION AND THE WAVELENGTH OF REFRACTORINESSIN THE RABBIT VENTRICLE

The effects of rate, premature beats, potassium, temperature,epinephrine and lidocaine

2.1. INTRODUCTION

Small reentrant circuits either within atrial (Allessie et al., 1976, 1977, 1984) or ventricular myocardium (Janse et al., 1980, Wit et al., 1982, 1987, Allessie et al., 1987, Dillon et al., 1988) around a functionally determined area of conduction block may play an important role in the pathogenesis of atrial or ventricular arrhythmias (El-Sherif et al., 1977b, 1981, 1983, Wit et al., 1987, Dillon et al., 1987). In contrast to the fixed pathway during circus movement tachycardias involving a gross anatomical obstacle (Rosenblueth and Garcia Ramos, 1947, Page et al., 1983, Frame et al., 1986) both the size and rate of reentrant tachycardias involving a functionally determined area of conduction block are completely determined by the electrophysiological properties of the myocardium (Allessie et al., 1977). The minimal perimeter of such a circuit will be equal to the length of the excitation wave (refractory period x conduction velocity, Allessie et al., 1977). When the wavelength is shortened either by depression of conduction velocity or by shortening of the refractory period, the size of the circuit will decrease. On the other hand a lengthening of the wavelength will result in an increase of the size of the circuit (Smeets et al., 1986). A short wavelength was found to correlate with a high vulnerability to reentrant arrhythmias either in isolated rabbit atrium or in conscious dogs (Smeets et al., 1986, Rensma et al., 1988). The wavelength of refractoriness emerged to be a sensitive and specific index of the susceptibility of the heart to atrial arrhythmias with an overall predictive power of 75% (Rensma et al., 1988).

There are some important morphological differences between atrium and ventricle which makes it difficult to extrapolate the wavelength concept to ventricular arrhythmias. In contrast to the more or less random fiber orientation in the atria, the ventricular cells are organized more in parallel to each other (Sommer and Dolber, 1979). Ventricular cells have an average diameter of 10 to 20 μ and a length ranging from 60 to 100 μ and are coupled to each other by nexuses. The nexuses form almost always end-to-end connections between the cells, although sometimes side-to-side connections are found (Sommer, 1983). Due to the different contribution of the cytoplasmic resistance and the nexus resistance to the effective axial resistance measured along and perpendicular to the fiber direction (Spach et al., 1979, 1981), the axial resistance is about nine times larger perpendicular to the fiber direction than parallel to the fiber orientation (Clerc, 1976). As a consequence, conduction parallel to the fiber orientation is about three times faster than perpendicular to the fiber orientation (Clerc, 1976, Spach et al., 1981, 1982b). Due to the anisotropic distribution of conduction velocity, the wavelength varies with different directions of impulse propagation. A short wavelength will be found during slow transverse impulse conduction, while in the same area the wavelength will be long during longitudinal conduction. Because of anisotropy in passive electrical properties, variations in nexus resistances and modifications of the active membrane properties may have different effects on longitudinal and transverse conduction velocity (Spach et al., 1981, 1982b, 1987, Tsuboi et al., 1985, Kadish et al., 1986, Delmar et al., 1987). Consequently the ratio between the longitudinal and transverse wavelength will be changed by these modifications.

The aim of the present study was to measure the longitudinal and transverse wavelength in the intact Langendorff perfused rabbit left ventricle and to evaluate the effects of heart rate, premature beats, potassium, temperature, epinephrine and lidocaine.

2.2. METHODS

Flemish rabbits (n = 35) of both sexes weighing between 4.5 and 5.5 kg were used in this study. After heparinization (1000 I.U/I.V.) the animals were killed by a cervical dislocation. The thorax was opened by a midsternal incision and the heart was rapidly removed and placed in cold perfusion fluid (10^{o}C). The aorta was cannulated and the heart was connected to a Langendorff perfusion system. The coronary arteries

were perfused with a perfusion pressure of 50 mm Hg, resulting in a flow of 35-45 ml/min. The millimolar composition of the perfusion fluid was: NaCl 130; $NaHCO_3$ 20.1; KCl 5.6; $CaCl_2$ 2.2; $MgCl_2$ 0.6; NaH_2PO_4 1.2 and glucose 12. The solution was saturated with a mixture of 95% O_2 and 5% CO_2. pH was 7.35, and temperature was kept at 37°C ± 0.5. Drugs could be administered to the perfusion fluid via the aorta canule using a infusion pump (Razel A-99).

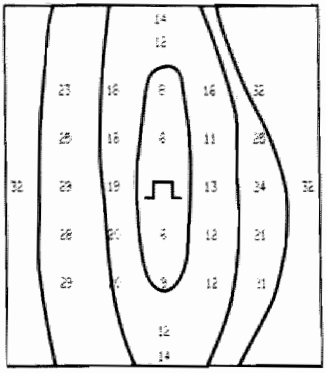
Recording and Stimulation.

To measure conduction velocities and local refractory periods at the epicardial surface of the left ventricle, a high resolution multiterminal electrode was used containing 30 single electrodes (silver wires diameter 0.3 mm) at regular distances of 2 mm (figure 1). Programmed electrical stimulation was performed with a programmable constant current stimulator delivering square pulses of 2 msec duration and 2-4 times diastolic threshold. The hearts were stimulated through a central pair of electrodes. Unipolar electrograms were recorded using the stainless steel cannula in the aorta as indifferent electrode. The recorded electrograms were fed into 30 individual amplifiers (bandwidth 2-400 Hz) and displayed in groups of eigth on two Textronix 5103N oscilloscopes.

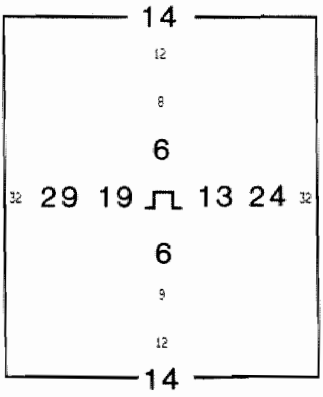
Measurement of refractory period.

Local refractory periods were determined by programmed electrical stimulation. After each 15 th beat a single premature stimulus (4 times diastolic threshold) was applied to the stimulating electrodes in the center of the multiple recording electrode. The functional refractory period was defined as the shortest possible interval between a regular impulse and a premature beat, at which a propagated impulse was present (shortest V1-V2 interval). This V1-V2 interval was measured at a recording electrode close to the site of stimulation.

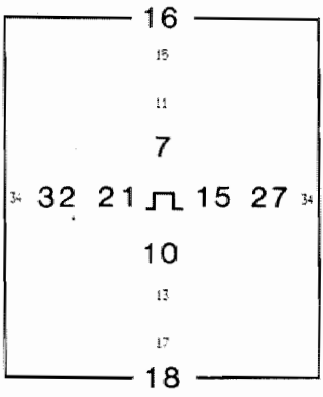
The maximal pacing rate (e.g. the shortest possible pacing rate) was determined by progressive shortening of the pacing interval until the stimulus was blocked either at the stimulus site or at a distal electrode. The maximal pacing rate (Fmax) was defined as the shortest pacing interval which resulted in a 1:1 propagated response.

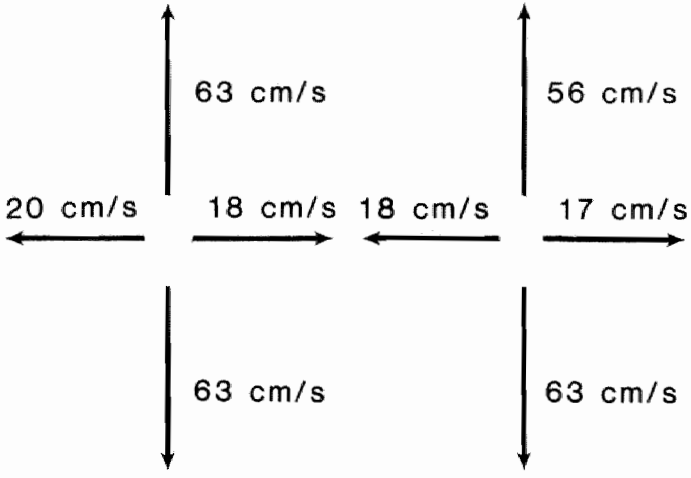


BASIC BEAT



PREMATURE BEAT





To accurately measure the refractory period, an electrogram recorded near the site of stimulation was fed into a transient recorder (Datalab DL901). From this transient recorder, activation times could be read with an accuracy of 1 ms.

Measurement of conduction velocity.

The conduction velocity of the cardiac impulse was determined in different directions using the high resolution mapping electrode (figure 1). The heart was stimulated through a pair of stimulating electrodes located in the center of the probe. Electrograms were monitored from 4 rows of electrodes, forming a cross with the central stimulating electrodes. The long axis of the electrode was positioned parallel to the epicardial fiber direction by looking for the highest amplitude and slope of the electrograms recorded along the long axis together with the shortest activation time between the proximal and distal electrodes (Kleber et al, 1986). After proper alignment conduction velocity in the longitudinal (V_L) and transverse (V_T) direction can be calculated from the differences in activation time along the rows of electrodes. Along the longitudinal axis of the probe the distance over which the conduction velocity was measured was 5 mm, whereas in the transverse direction only the first two recording electrodes (distance 2 mm) could be used, because at larger distances transverse conduction was interrupted by epicardial breakthrough (figure 1). The V_L / V_T ratio was taken as an index of the degree of anisotropy of the epicardium.

Figure 2.1. Measurement of conduction velocity. An activation map recorded during pacing at a cycle length of 350 ms from the central pair of electrodes with the high resolution mapping electrode is shown. An ellipsoid wavefront, with slow conduction perpendicular to the fiber direction and fast conduction parallel to the fiber direction was recorded. The conduction velocity of the cardiac impulse was determined in different directions. Electrograms were monitored from 4 rows of electrodes, forming a cross with the central stimulating electrodes. The long axis of the electrode was positioned parallel to the epicardial fiber direction. After proper alignment conduction velocity in the longitudinal (V_L) and transverse (V_T) direction can be calculated from the differences in activation time along the rows of electrodes. Along the longitudinal axis of the probe the distance over which the conduction velocity was measured was 5 mm, whereas in the transverse direction only the first two recording electrodes (distance 2 mm) could be used, because at greater distances transverse conduction was interrupted by epicardial breakthrough

Measurement of the wavelength.

The wavelength of the cardiac impulse is defined as the distance travelled by the impulse during the time the myocardium restores its excitability (Wiener and Rosenblueth, 1946, Smeets et al, 1986, Rensma, 1987, Rensma et al., 1988). It can be calculated by multiplying conduction velocity and the functional refractory period.

WAVELENGTH(mm)
=
CONDUCTION VELOCITY(cm/s) x REFRACTORY PERIOD(ms)

Because of the differences between longitudinal and transverse conduction, the wavelength has to be calculated in both directions. The wavelength of a premature beat was measured by adding a second shortest possible coupled extrastimulus to the stimulation protocol. When the hearts were paced at their maximal pacing frequency (F_{max}) the wavelength was defined as the product of conduction velocity and the interval of Fmax.

Statistical analysis.

The level of significance between groups was calculated using Students T-Test. The level of significance was taken as $p < 0.05$.

2.3. RESULTS

The effects of rate and premature beats.

To investigate the effects of rate and premature beats on the wavelength and the degree of anisotropy in the ventricles, the hearts were paced at different pacing intervals and premature stimuli were applied at different degrees of prematurity and at different pacing intervals.

In figure 2.2A and table 2.IA the effects of rate on refractoriness, conduction velocity and wavelength as measured in 18 hearts are given. The ventricles were paced

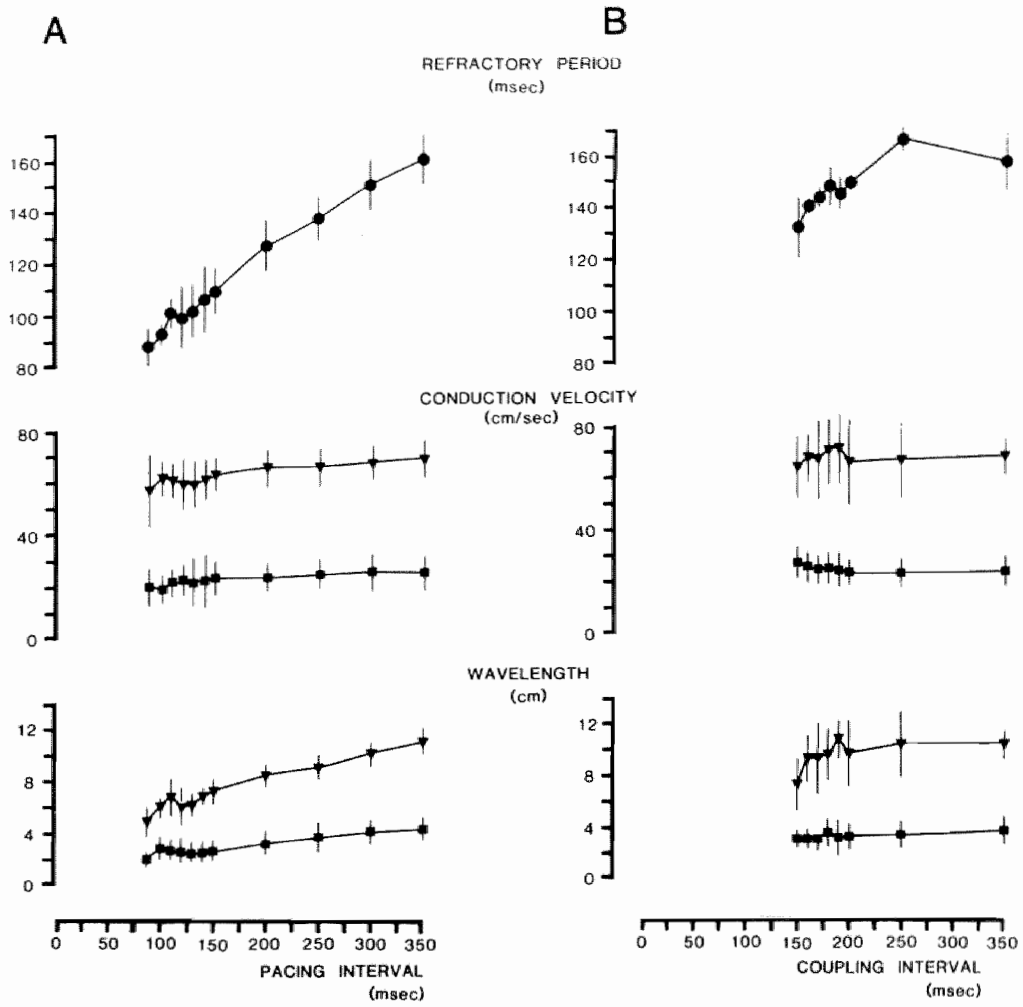


Figure 2.2. Panel A: The effects of incremental pacing on refractory period, conduction velocity and wavelength of regularly driven impulses. The mean values (n = 18) and standard deviations of the refractory period (●), the longitudinal (▼) and transverse conduction velocity (■), and the longitudinal and transverse wavelength are given as a function of the pacing interval. Because of a strong shortening of the refractory period and a reduction of both the longitudinal and the transverse conduction velocity, the wavelength in both directions is shortened with about 55 %. In panel B the effects of the degree of prematurity during regular pacing at a pacing interval of 350 ms on the wavelength is given. Both the longitudinal and transverse wavelength shortened respectively with 20 % and 11 %

Table 2.I

A

Effects of pacing frequency on the wavelength.

V1V1 ms	V1V2 ms	V_L cm/s	V_T cm/s	V_L/V_T	WL_L cm	WL_T cm
350	158 ±12	68 ± 7	24 ± 6	2.9 ±0.9	10.5 ±1.1	3.9 ±1.1
200	128 ±10 **	65 ± 7 ns	23 ± 6 ns	2.9 ±0.9 ns	8.2 ±1.1 **	3.0 ±1.1 **
150	109 ± 9 **	64 ± 7 *	22 ± 7 ns	3.0 ±1.0 ns	7.0 ±1.0 **	2.5 ±0.9 **
F_{max}	87 ± 8 **	57 ±15 *	21 ± 8 ns	2.9 ±1.1 ns	4.7 ±1.2 **	1.7 ±0.7 **

B

Effects of prematurity (V1-V1 = 350 ms).

V1V2 ms	V2V3 ms	V_L cm/s	V_T cm/s	V_L/V_T	WL_L cm	WL_T cm
350	158 ±12	68 ± 7	24 ± 6	2.9 ±0.9	10.5 ±1.1	3.9 ±1.1
250	166 ± 5 ns	66 ±15 ns	23 ± 6 ns	2.9 ±1.3 ns	10.5 ±2.5 ns	3.6 ±1.1 ns
200	150 ± 1 *	66 ±17 ns	24 ± 5 ns	3.1 ±1.3 ns	9.8 ±2.5 ns	3.4 ±1.0 ns
170	144 ± 4 *	67 ±15 ns	25 ± 6 ns	2.9 ±0.8 ns	9.4 ±2.7 ns	3.2 ±0.9 ns
160	141 ± 3 *	68 ± 9 ns	26 ± 6 ns	2.6 ±0.7 ns	9.4 ±1.8 ns	3.2 ±0.8 ns
150	132 ±12 **	64 ±12 ns	26 ± 6 ns	2.4 ±0.6 ns	7.4 ±2.0 ns	3.2 ± 1.0 ns

C

The effects of rate on the wavelength of the earliest premature beat.

V1V1 ms	V1V2 ms	V_L cm/s	V_T cm/s	V_L/V_T	WL_L cm	WL_T cm
350	140 ±10	62 ±11	27 ± 6	2.5 ±0.9	9.3 ±2.1	3.3 ±0.8
200	104 ± 9 **	60 ± 9 ns	23 ± 6 ns	2.7 ±0.9 ns	6.4 ±1.6 **	2.4 ±0.8 **
150	93 ±10 **	57 ±10 *	22 ± 7 ns	2.8 ±0.8 ns	5.7 ±1.6 **	2.2 ±1.0 **

* $p<0.05$ ** $p<0.005$

with cycle lengths ranging from 350 ms to the shortest possible pacing interval (87 ms). The refractory period during pacing at a moderate rate of 350 ms cycle length was 158 ms. The refractory period shortened with about 45 % when the pacing interval was gradually shortened from 350 ms to the shortest pacing interval ($p<0.005$). The longitudinal conduction velocity decreased from 68 cm/s with about 16% to 57 cm/s ($p<0.05$). The transverse velocity decreased from 24 cm/s with about 12% to 21 cm/s (ns). The degree of anisotropy (the ratio between V_L and V_T) was 2.9 and did not change significantly. Because of the shortening of the refractory period and the slowing of conduction, at higher heart rates the wavelength shortened from 10.5 cm to 4.7 cm (-56%, $p<0.005$) during longitudinal conduction and from 3.9 cm to 1.7 cm (-54%, $p<0.005$) during transverse propagation.

The effects of the premature beats on refractoriness, conduction velocity and wavelength during pacing at a regular rate of 350 ms are given in table 2.IB and figure 2.2B ($n=7$). The coupling interval of the premature stimulus was varied between 150 ms and 250 ms. The refractory period of a premature beat decreased with about 21%, from 166 ms to 132 ms ($p<0.005$) when the coupling interval was shortened from 250 to 150 ms. The longitudinal conduction velocity varied between 68 cm/s and 64 cm/s (ns) and transverse conduction between 23 cm/s and 26 cm/s (ns). The degree of ani-

sotropy was 2.9 during late premature beats and 2.4 during early premature beats (ns). Because of the shortening of the refractory period both the longitudinal and the transverse wavelength of early premature beat decreased from 10.5 cm to 7.4 cm (-20%) and from 3.9 cm to 3.2 cm (-11%).

The effects of the pacing rate on the wavelength of the earliest premature beat and the degree of anisotropy is summarized in table 2.IC ($n = 10$). The refractory period of the earliest premature impulse (the shortest V2-V3 interval) shortened from 140 ± 10 ms during pacing with 350 ms, to 93 ms during a basic cycle lenght of 150 ms (-44%, $p < 0.005$). The longitudinal conduction velocity of the earliest premature beat decreased from 62 cm/s to 57 cm/s (-8%, $p < 0.05$) and the transverse velocity from 27 cm/s to 22 cm/s (-18%, ns). The degree of anisotropy increased from 2.5 to 2.8 (+12%, ns). The longitudinal wavelength shortened from 9.3 cm to 5.7 cm (-39%, $p < 0.005$) and the transverse wavelength from 3.3 cm to 2.2 cm (-33%, $p < 0.005$).

The effects of potassium.

The effects of potassium were studied during pacing at a pacing rate of 350 ms ($n = 5$) and during maximal pacing ($n = 5$). The potassium concentration was increased gradually starting from 5.6 mM. Every 15 minutes a sample of the perfusion fluid was taken and the electrophysiological parameters were measured. After the experiment the exact potassium concentration was determined in the samples of the perfusion fluid.

In figure 2.3 and table 2.II the effects of increasing concentrations of extracellular potassium are given. During pacing at 350 ms interval the refractory period and the conduction velocities were not altered as long as the potassium concentration remained lower than 8.0 mM. When the concentration was further increased the refractory period lengthened from 160 to 252 ms (+58%, $p < 0.05$). Longitudinal conduction was strongly affected and slowed down from 66 to 30 cm/s (-55%, $p < 0.05$). At the same time the transverse conduction velocity was not changed significantly (24 versus 22 cm/s). As a result the degree of anisotropy sharply decreased from 2.8 during control to 1.5 during hyperkalemia (-44%, $p < 0.05$). In a longitudinal direction the slowing of conduction clearly exceeded the lengthening of the refractory period and consequently the longitudinal wavelength decreased from 10.5 to 7.9 cm (-25%, $p < 0.05$). In a transverse direction conduction velocity was not altered and the prolongation of

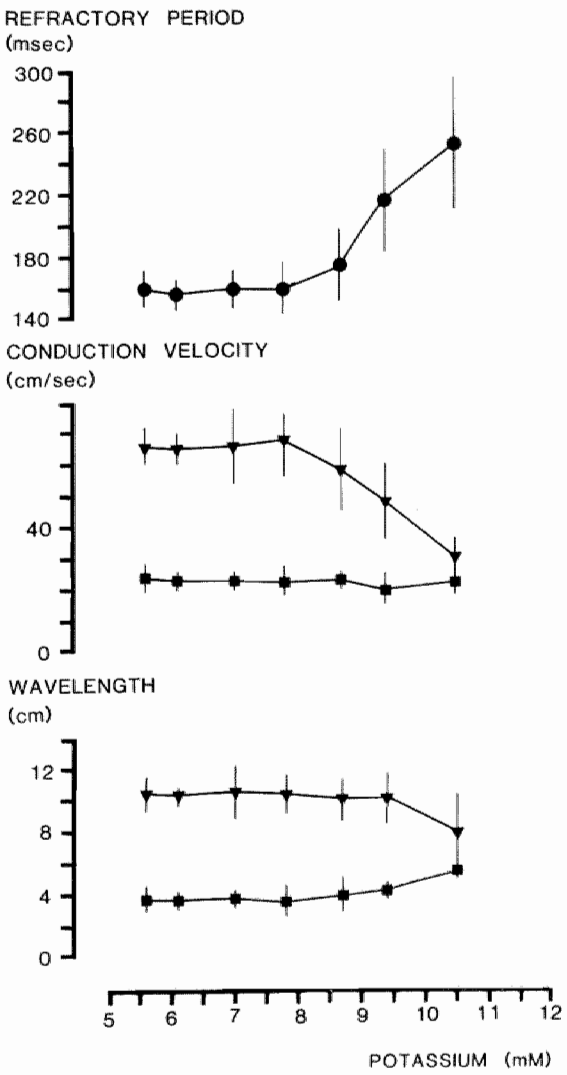


Figure 2.3. The effects of extracellular potassium concentration on the wavelength during regular pacing (350 ms cycle length, n = 5). The longitudinal wavelength shortened from 10.5 ± 1.0 cm to 7.9 ± 2.4 cm due to a slowing of the longitudinal conduction velocity from 64 ± 6 cm/s to 32 ± 4 cm/s. The transverse wavelength however prolonged from 3.8 ± 0.8 to 5.5 ± 0.5 cm because the transverse conduction velocity is only slightly depressed while the refractory period lengthened from 160 ± 11 ms to 252 ± 43 ms.

Table 2.II

A

The effects of extracellular potassium during slow pacing (V1V1 350ms).

Potassium mM	FRP ms	V_L cm/s	V_T cm/s	V_L/V_T	WL_L cm	WL_T cm
5.6	160 ±11	66 ± 6	24 ±5	2.8 ±0.6	10.5 ±1.0	3.8 ±0.8
7.0	160 ±17 ns	67 ±11 ns	23 ±4 ns	3.0 ±0.9 ns	10.4 ±1.2 ns	3.6 ±1.0 ns
9.4	216 ±33 *	48 ±11 *	20 ±5 ns	2.5 ±0.5 ns	10.0 ±1.6 ns	4.2 ±0.6 ns
10.5	252 ±43 *	30 ± 6 *	22 ±4 ns	1.5 ±0.5 ns	7.9 ±2.4 *	5.5 ±0.5 *

B

The effects of extracellular potassium during maximal pacing.

Potassium mM	Fmax ms	V_L cm/s	V_T cm/s	V_L/V_T	WL_L cm	WL_T cm
5.6	79 ± 8	57 ±14	21 ±6	2.7 ±1.1	4.5 ±1.1	1.8 ±0.6
7.0	124 ±28 *	45 ±15 ns	20 ±6 ns	2.4 ±1.1 ns	5.3 ±1.1 ns	2.4 ±0.7 ns
9.4	218 ±44 **	35 ±16 *	17 ±3 ns	1.8 ±0.7 *	7.0 ±3.0 *	3.4 ±0.6 *
10.5	255 ±50 **	29 ± 7 *	19 ±4 ns	1.6 ±0.7 *	7.4 ±2.0 *	5.2 ±1.1 *

* $p<0.05$ ** $p<0.005$

the refractory period thus resulted in a clear prolongation of the transverse wavelenght from 3.8 to 5.5 cm (+44%, $p<0.05$).

During potassium administration (table 2.IIB) the shortest possible pacing interval gradually increased (from 79 to 255 ms (+179%, $p<0.005$). Longitudinal conduction velocity during Fmax slowed from 57 to 29 cm/s (-49%, $p<0.05$) and the transverse velocity from 21 to 19 cm/s (-10%, ns). This resulted in a marked decrease of the degree of anisotropy from 2.7 to 1.6 (-41%, $p<0.05$). Because the lengthening of the shortest pacing interval was not fully counteracted by the slowing of conduction, both in the longitudinal and in the transverse direction the wavelength increased. During Fmax the longitudinal wavelength prolonged from 4.5 to 7.4 cm (+64%, $p<0.05$) and the transverse wavelength from 1.8 cm to 5.2 cm (+188%, $p<0.05$).

The effects of temperature.

The effects of temperature were studied during pacing at a cycle length of 950 ms and during the maximum pacing frequency ($n=5$). In figure 2.4 and table 2.III the mean values of the refractory period, conduction velocity and wavelength are given. Changes in temperature had pronounced effects on both conduction velocity and refractory period. When during pacing at a constant cycle length of 950 ms the temperature was lowered from 37 °C to 23 °C the refractory period lengthened from 185 ms to 441 ms (+24%, $p<0.005$). At the same time longitudinal conduction velocity slowed down from 64 cm/s to 32 cm/s (-50%, $p<0.05$) and the transverse velocity from 29 cm/s to 11 cm/s (-62%, $p<0.05$). During cooling the V_L/V_T ratio increased from 2.3 to 2.8 (+21%, ns).

As can be seen from figure 4 the effects of cooling on the wavelength are bifasic, both in a longitudinal and a transverse direction. When the temperature was lowered from 37 °C to 29 °C, the wavelength first increased from 11.8 cm to 15.0 cm (+27%, $p<0.05$) and from 5.4 cm to 6.8 cm (+26%, $p<0.005$) respectively. In this temperature range the lengthening of the refractory period was greater than the decrease in conduction velocity. However when the temperature was further lowered from 29 ° C to 23 °C the wavelength shortened again to 13.8 cm (+16%, ns) and 5.0 cm (-7%, ns) because the conduction velocity was stronger affected than the refractory period.

In table III B the effects of cooling on the wavelength are given during pacing at the maximum pacing frequency. The shortest possible pacing interval increased from

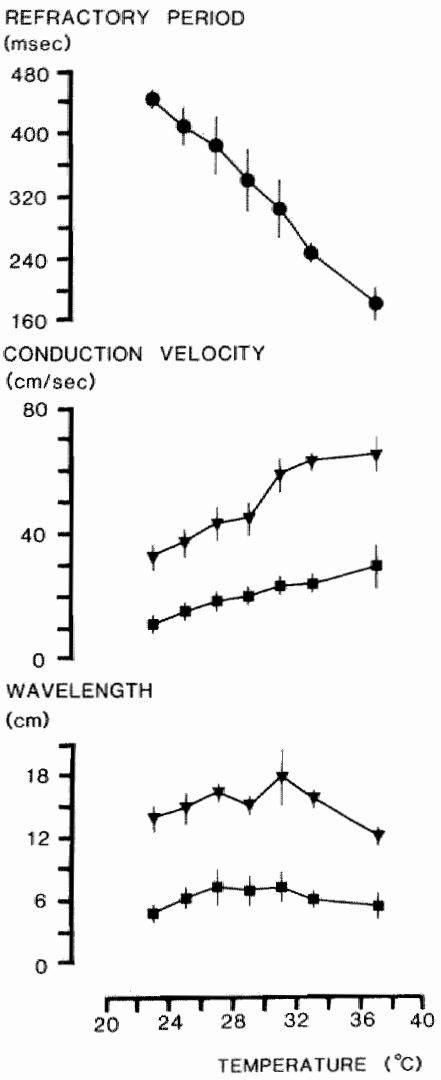


Figure 2.4. The influence of temperature on the wavelength during slow regular pacing (950 ms, (n = 6). When the temperature was lowered to 29 °C, both the longitudinal and the transverse wavelength lengthened (respectively from 11.9 ± 1.1 cm to 15.1 ± 0.5 cm and from 5.4 ± 1.4 cm to 6.8 ± 1.5 cm). A further lowering of the temperature to 23 °C resulted in a shortening of the longitudinal and the transverse wavelength. However both the longitudinal and the transverse wavelength were not significantly altered compared with the values determined at 37 °C.

Table 2.III

A

The effects of Temperature (V1-V1 950 ms).

Temp °C	Frp ms	V_L cm/s	V_T cm/s	V_L/V_T	WL cm	WL_T cm
37	185 ±16	64 ±6	29 ±7	2.3 ±0.6	11.9 ±1.1	5.4 ±1.4
29	342 ±39	45 ±5	20 ±3	2.3 ±0.4	15.1 ±0.5	6.8 ±1.5
	**	**	*	ns	*	**
25	408 ±23	37 ±4	15 ±1	2.4 ±0.3	14.8 ±1.6	6.2 ±0.4
	**	**	*	ns	ns	ns
23	441 ±15	32 ±4	11 ±1	2.8 ±0.5	13.8 ±1.2	5.0 ±0.5
	**	**	*	ns	ns	ns

B

The effects of Temperature (F_{max}).

Temp °C	Frp ms	V_L cm/s	V_T cm/s	VL/V_T	WL_L cm	WL_T cm
37	110 ± 3	58 ±11	27 ±2	2.1 ±0.4	6.4 ±1.3	3.0 ±0.3
29	191 ±15	40 ± 7	17 ±4	2.4 ±0.3	7.6 ±1.2	3.2 ±0.6
	**	*	*	ns	ns	ns
25	236 ± 1	33 ± 5	11 ±3	3.0 ±0.4	7.7 ±1.2	2.7 ±0.7
**	**	*	ns	ns	ns	
23	280 ±12	29 ± 4	9 ±3	3.4 ±0.6	8.0 ±1.0	2.4 ±0.6
	**	**	*	ns	ns	ns

* $p<0.05$ ** $p<0.005$

110 ms to 322 ms (+193%, $p<0.005$) when the temperature was lowered from 37 °C to 23 °C. The longitudinal conduction velocity decreased from 58 cm/s to 29 cm/s (-50%, $p<0.005$) and transverse conduction velocity from 27 cm/s to 9 cm/s (-67%, $p<0.005$) resulting in an increase of the V_L/V_T ratio from 2.1 to 3.4 (+62%, ns). During rapid pacing the decrease of the longitudinal conduction velocity was exceeded by the lengthening of the refractory period resulting in an lengthening of the longitudinal wavelength from 6.4 cm to 8.0 cm (+25%, ns). However because of the strong slowing of conduction velocity in a transverse direction the transverse wavelength actually decreased from 3.0 cm to 2.4 cm (-20%, ns). This opposite effect of cooling on the wavelength in a longitudinal and transverse direction resulted in an exaggeration of the difference in wavelength in different directions. Whereas during normal temperature the longitudinal wavelength was 2 times as long as the transverse wavelength, at 23 °C the longitudinal wavelength was about four times as long as the transverse wavelength.

The effects of epinephrine.

The effects of epinephrine ($n = 5$) on refractoriness, conduction velocity and wavelength are summarized in table 2.IV and figure 2.5A. Because it is known that the effectiveness of epinephrine increases when the pacing frequency is increased the preparations were paced at a cycle length of 200 ms. The concentration of epinephrine was increased in steps from 0.9x10-7M to 4.5x10-7M. The refractory period shortened from 148 ms during control to 123 ms (-17%, $p<0.05$) at the highest concentration of epinephrine. The longitudinal conduction velocity increased from 52 cm/s to 57 cm/s (+10%, ns), and the transverse conduction velocity increased from 19 cm/s, to 21 cm/s (+11%, ns). The degree of anisotropy was not changed by epinephrine. The longitudinal wavelength decreased from 7.7 cm to 7.0 cm (-7%, ns) and from 2.9 cm to 2.5 cm (-14%, ns) perpendicular to the fiber direction.

The effects of lidocaine.

Figure 2.5B and table 2.V give the effects of different concentrations of lidocaine ($n=5$) on conduction velocity, refractory period and wavelength. Because of the rate dependent properties of lidocaine the hearts were paced at a relatively high rate (200

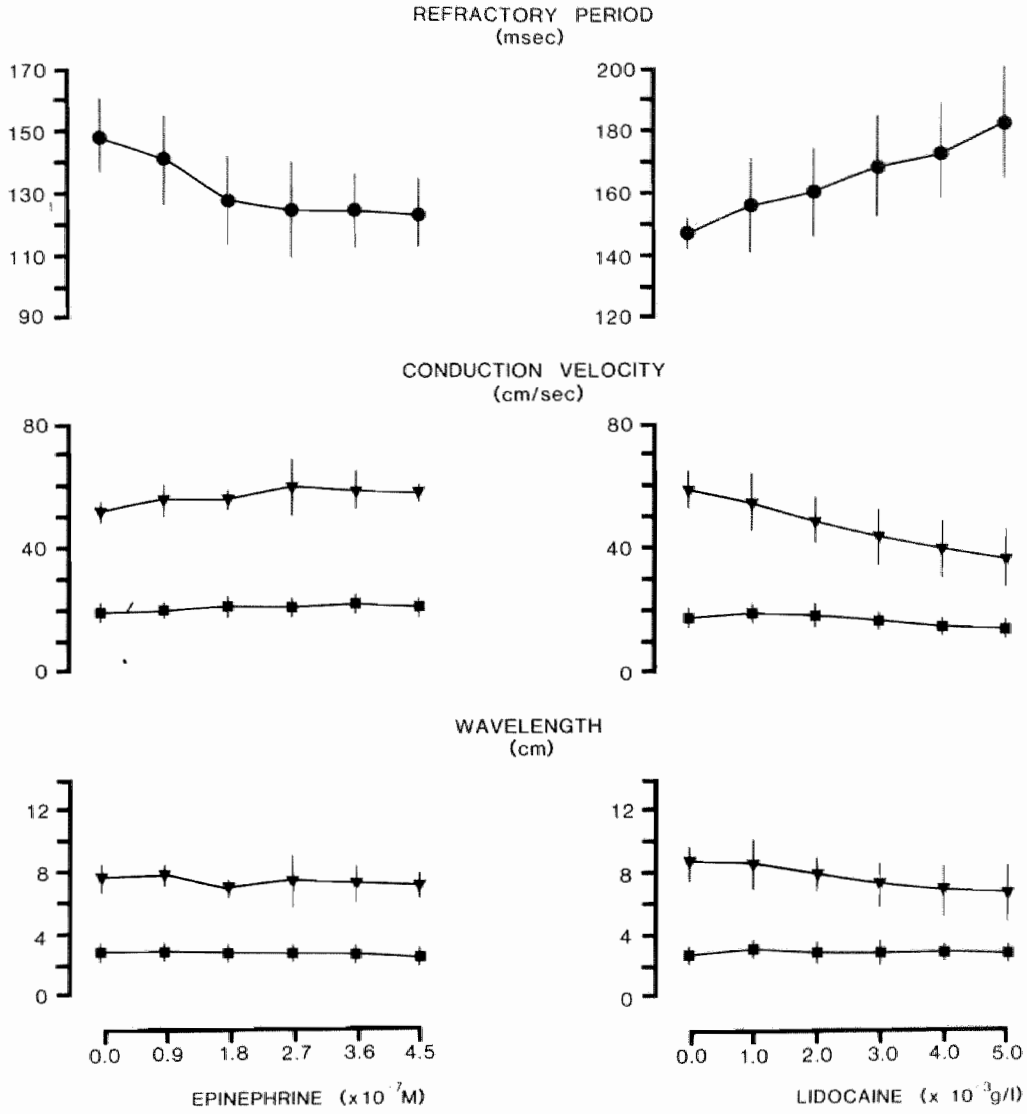


Figure 2.5. The effects of increasing concentrations of epinephrine (n = 5, panel A) and Lidocaine (n = 5, panel B) on the wavelength during regular pacing with a cycle length of 200 ms. During epinephrine the wavelength was not changed because the shortening of the refractory period (from 148 ± 12 ms to 123 ± 11 ms) was abolished by a slight increase of both the longitudinal (from 52 ± 3 cm/s to 57 ± 3 cm/s) and the transverse conduction velocity (from 19 ± 2 cm/s to 21 ± 1 cm/s). During lidocaine the refractory period increased gradually from 146 ± 4 ms during control to 182 ± 18 ms at the highest concentration ($5x10^{-3}$ g/l). Longitudinal conduction velocity slowed from 58 ± 5 cm/s to 36 ± 9 cm/s and the longitudinal wavelength shortened from 8.5 ± 1.0 cm to 6.5 ± 1.8 cm. The transverse wavelength was not altered and remained 2.7 ± 0.3 cm.

Table IV

The effects of Epinephrine (V1V1 = 200 ms).

Epineph. M	Frp ms	V_L cm/s	V_T cm/s	V_L/V_T	WL_L cm	WL_T cm
control	148 ±12	52 ±3	19 ± 2	2.7 ± 0.2	7.7 ±0.8	2.9 ±0.2
$2.7x10^{-7}$	124 ±15 *	59 ±9 ns	21 ± 2 ns	2.9 ±0.6 ns	7.3 ±1.7 ns	2.6 ± 0.4 ns
$4.5x10^{-7}$	123 ±11 *	57 ±3 ns	21 ±1 ns	2.8 ±0.3 ns	7.0 ±0.8 ns	2.5 ±0.3 ns

* $p<0.05$ ** $p<0.005$

Table V

The effects of Lidocaine (V1V1 = 200 ms).

Lidocaine g/ml	Frp ms	V_L cm/s	V_T cm/s	V_L/V_T	WL_L cm	WL_T cm
Control	146 ± 4	58 ± 5	19 ± 3	3.1 ±0.5	8.5 ±1.0	2.7 ±0.3
$3.0x10^{-6}$	168 ±16 **	43 ±9 *	16 ±3 *	2.8 ±0.8 ns	7.1 ±1.4 ns	2.8 ±0.8 ns
$4.0x10^{-6}$	173 ±15 **	39 ±9 *	14 ±3 **	2.8 ±0.7 ns	6.7 ±1.6 ns	2.8 ±0.7 ns
$5.0x10^{-6}$	182 ±18 **	36 ±9 *	14 ±3 **	2.7 ±0.7 ns	6.5 ±1.8 ns	2.7 ±0.7 ns

* $p<0.05$ ** $p<0.005$

ms cycle length) (Buchanan et al., 1985). When the lidocaine concentration was increased stepwise from 0 to 5.10^{-6} g/ml, the refractory period increased from 146 ms to 182 ms (+20%, $p<0.005$). The longitudinal conduction velocity decreased from 58 cm/s to 36 cm/s (-38 %, $p<0.05$) and transverse conduction velocity decreased from 19 cm/s, to 14 cm/s (- 26%, $p<0.005$). Because longitudinal conduction was affected more by lidocaine than transverse conduction, lidocaine caused a decrease of the degree of anisotropy from 3.1 to 2.7 (-13%, ns). The wavelength measured along the longitudinal axis decreased from 8.6 cm to 6.5 cm (-24%, ns), whereas the transverse wavelength was not altered by lidocaine and remained 2.7 cm.

2.4. DISCUSSION

The wavelength of refractoriness and the spatial inhomogeneity in conduction have shown to be key factors in the induction of reentrant arrhythmias (Smeets et al.,1986, Rensma et al., 1988, Lammers et al., 1988). In addition in recent years several investigators have reported that anisotropy plays an important role in the occurrence of local conduction block and the initiation of reentry (Spach et al., 1981, 1986, 1987, Wit et al., 1987, Dillon et al., 1988). First the effects on the degree of anisotropy (the ratio between longitudinal and transverse conduction velocity) will be discussed. The different groups are grouped on their effects on the degree of anisotropy and regrouped on their effects on the wavelength of refractoriness.

Effects of rate.

In 1976 Clerc demonstrated that the axial resistance is 9.4 times larger in a transverse than in a longitudinal direction. Because conduction velocity is inversely related to the square root of the axial resistance (Hodgkin and Huxley, 1952), conduction in a tranverse direction is about three times slower than in a longitudinal direction (Clerc 1976, Spach et al.,1981, 1982b, Tsuboi et al., 1985, Delmar et al.,1987). The results of our control studies are in agreement with this. During pacing with a pacing interval of 350 ms the longitudinal conduction velocity was 68 cm/s and the transverse conduction velocity 24 cm/s resulting in a V_L/V_T ratio of 2.9. Due to the different contribution of the cytoplasmic resistance and the nexus resistance to the effective axial resistance

measured parallel and perpendicular to the fiber direction (Spach et al., 1979, 1981), an increase of the nexus resistance will slow down transverse conduction to a greater extent than longitudinal conduction resulting in an increase of the V_L/V_T ratio. Spach et al. (1982), Tsuboi et al. (1985) and Delmar et al. (1987) showed that when the nexus resistance was increased by rapid pacing or heptanol administration transverse conduction slowed to a greater extent than longitudinal conduction resulting in an increase of the V_L/V_T ratio. The increase of the nexus resistance during rapid pacing was explained, by assuming that during rapid pacing the calcium concentration rises due to an increased sodium-calcium exchange (Bredikis et al., 1981, Cohen et al., 1982, Spach et al., 1982, DeMello, 1976, 1983). This was supported by the finding that verapamil partially abolished the slowing of the transverse conduction velocity by blocking the calcium inward current (Tsuboi et al., 1985, Delmar et al., 1987). In our study however the V_L/V_T ratio was not altered during rapid pacing, longitudinal and transverse conduction being slowed down to the same extent. This difference in our results and the data of Spach et al.(1982), Tsuboi et al.(1985) and Delmar et al.(1987) might be explained by the different preparations used in these studies. In most studies isolated sheets of myocardium and papillary muscle preparations superfused in a tissue bath are used to study anisotropic conduction (Clerc, 1977, Spach et al., 1982b, 1986, Tsuboi et al., 1985, Kadish et al., 1986, Delmar et al., 1987). To maintain stable conduction, such experiments had to be carried out at reduced temperatures (25-30 °C) or within a limited range of pacing frequencies. Under normothermic conditions the conduction velocity of in vitro preparations is strongly rate dependent and conduction block occurs at a lower pacing frequency than in a perfused heart (Spach et al., 1982b, Tsuboi et al., 1985, Delmar et al., 1987). In the intact Langendorff perfused rabbit left ventricle as used in our studies, the maximal pacing frequency is 2-3 times higher than in superfused preparations. At the maximal pacing rate conduction velocity slowed down only 15 % and stable conditions could be maintained for more than eight hours without any significant change of the electrophysiological properties. It can thus be concluded that the results obtained by using a Langendorff perfused heart are

Effects of premature beats and potassium

When the membrane properties of anisotropic myocardium are depressed homogeneously the V_L/V_T ratio should not be altered (Spach et al.,1981, 1982b). In agree-

ment with this Spach et al. (1982b), Tuboi et al., (1985), and Delmar et al. (1987) demonstrated that the V_L/V_T ratio was not changed by premature beats or increased extracellular potassium concentration. In our study we found that conduction was not changed during premature beats and the degree of anisotropy was not altered.

However in contrast with the results of Tsuboi et al. (1985) we demonstrated that when the extracellular potassium concentration was increased to 10.5 mM, longitudinal conduction was slowed down to a greater extent than transverse conduction, both during regular pacing and during pacing at the maximal pacing frequency. The V_L/V_T ratio decreased, from 2.8 to 1.5 during regular pacing (350 ms interval) and from 2.7 to 1.6 during pacing at the maximal pacing frequency. Dominquez and Fozzard (1970) showed that when the potassium concentration is elevated above 7.5 mM both conduction velocity and Vmax decreased and a linear relation between the Vmax of the action potential and the square of the conduction velocity was found (Dominquez and Fozzard, 1970, January and Fozzard, 1984, Buchanan et al., 1985, Kishida et al., 1979). Because it is known that the longitudinal V_{max} is depressed to a greater extent than the transverse Vmax (Tsuboi et al., 1986) the ratio between the longitudinal and transverse upstroke velocity (V_{max}) of the action potential decreased significantly. According to the results of Dominquez and Fozzard (1970) the decrease of the ratio between the longitudinal and transverse Vmax should thus be accompanied by a decrease of the V_L/V_T ratio which is in accordance with the results of our study.

Effects of cooling

It is known that during cooling the activity of the Na/K pump is depressed (Jack et al.,1975, Noble, 1975). Because the Na/K pump is necessary to maintain a low intracellular sodium concentration, cooling of the heart results in an increase of the intracellular sodium concentration. As a result of this increase of the sodium concentration, sodium will be exchanged by calcium leading to an increased intracellular calcium concentration (DeMello, 1976). The increase of the intracellular calcium concentration results in increase of the junctional resistance. Due to the different contribution of the junctional resistance to the effective axial resistance in anisotropic myocardium (Clerc, 1977, Spach et al., 1979, 1981) transverse conduction is slowed to a greater extent than the longitudinal conduction. Our results demonstrated that the V_L/V_T ratio increased from 2.3 to 2.8 during slow pacing (950 ms interval) and from 2.1

to 3.4 during pacing at a maximal pacing frequency. Due to the limited number of experiments (n=5) however this increase of the V_L/V_T ratio was not statistically significant.

Effects of epinephrine and lidocaine

Epinephrine increases the calcium inward current resulting in an increased intracellular calcium concentration (Noble, 1975). As a result of the increased intracellular calcium concentration it can be expected that the nexus resistance increases (DeMello, 1983). However epinephrine also stimulates the uptake of free intracellular calcium into internal membraneous organells such as mitochondria and sarcoplasmic reticulum. As a result of this a low intracellular free calcium concentration is maintained and the nexus resistance is not changed (Jack et al., 1975, Noble, 1975). Because epinephrine has no effects on the sodium inward current (Noble, 1975) the Vmax of the action potential will not be affected and conduction velocity will not change, which is in agreement with the results of the study of Antoni and Zerweck (1967). During epinephrine administration in our study conduction velocity was not changed and the degree of anisotropy remained 2.7.

Class I antiarrhythmic drugs which depress conduction velocity by blocking the fast sodium channels (Hoffman and Cranefield, 1960, Buchanan et al., 1985, Kadish et al., 1986, Spach et al., 1987), have been reported to exert different effects on longitudinal and transverse conduction. Kadish et al.(1986) demonstrated that during the administration of procainamide, longitudinal conduction was slowed down to a greater extent than tranverse conduction resulting in a decrease of the V_L/V_T ratio. This was explained by assuming a different drug binding capacity (Kadish et al., 1986, Spach et al., 1987). It is known that the amount of drug binding depends on the opening time of the fast sodium channels (Arnsdorf and Bigger, 1976, Buchanan et al., 1985). Spach and Kootsey (1985) showed that the opening time of the sodium channels is longer during longitudinal conduction than during transverse conduction resulting in a longer drug binding time during longitudinal conduction than during transverse conduction. In our study lidocaine administration resulted in a small and not significant decrease in the degree of anisotropy from 3.1 to 2.7. Despite the differences between class I A, B and C antiarrhythmica it can be expected that after administration these drugs

change the degree of anisotropy, e.g. the V_L/V_T ratio, because they all affect the upstroke velocity of the action potential by depressing the sodium inward current.

Tissue anisotropy and the wavelength of refractoriness.

Among other things the inducibility of reentrant arrhythmias is determined by the wavelength of refractoriness (wavelength = conduction velocity x functional refractory period, Smeets et al., 1986, Rensma et al., 1988). When the wavelength is shortened either by slowing of conduction or by shortening of the refractory period only a small area of conduction block will be needed to initiate reentry. A short wavelength was found to correlate with a high vulnerability to reentrant arrythmias either in isolated rabbit atrium and in conscious dogs (Smeets et al.,1986, Rensma et al., 1988). In the latter studies the wavelength of refractoriness was shown to be a sensitive and specific index of the susceptibility of the heart to atrial arrhythmias with an overall predictive power of 75%. Although in the present study we did not directly correlate the length of the excitation wave with the inducibility of ventricular arrhythmias the results seems to support the wavelength concept.

It is known from experimental (El-Sherif et al.,1977b, Dillon et al., 1988) and clinical studies (Wellens et al.,1972, 1974, Wellens, 1975a) that rapid ventricular pacing or premature stimuli may initiate reentrant ventricular arrhythmias. The observation that during incremental pacing and during premature beats the longitudinal and transverse wavelength shortened is in agreement with the enhanced risk of the initation of reentry. During incremental pacing when the pacing interval decreased from 350 ms to 85 ms, the shortening of the wavelength with 55% was caused by a shortening of the refractory period with 45% and a slowing of conduction with $\pm$15%. During premature beats the shortening of the wavelength was completely caused by a shortening of the refractory period (-21%), both the conduction velocity in a longitudinal and transverse direction not changing significantly.

The effects of potassium and cooling

Increased extracellular potassium depresses conduction and prolongs refractoriness in cardiac tissue (Fish et al., 1963). Conduction velocity and refractory period were affected to the same extent when the concentration was increased upto 7.0 mM

and the wavelenght was not changed. However when the potassium concentration was increased to 10.5 mM the wavelength was significantly changed. During regular pacing (350 ms pacing interval) the longitudinal wavelength (-25%) decreased because the lengthening of the refractory period (+58%) was less than the slowing of the conduction velocity (-55%). The transverse wavelength increased (+41%) due to the lengthening of the refractory period wheras transverse conduction was not changed. During pacing at the maximal pacing frequency both the longitudinal and tranverse wavelength lengthened (respectively with 64 % and 188 %) due to a 176% increase of the maximal pacing interval despite a slowing of longitudinal conduction (-49%) and transverse conduction (-10%). This lengthening of the wavelength during maximal pacing is in accordance with the known defibrillatory action of potassium infusion (Wiggers, 1953) and may explain that a single dose of high potassium administered during fibrillation or tachycardia in patients will terminate the arrhythmias, before the hearts become totally inexcitable (Grumbach et al., 1954).

It is a frequent observation that ventricular tachycardia, initiated in patients after a myocardial infarction sometimes could not be initiated anymore during cardiac surgery under hypothermia (Josephson and Wellens, 1984). If these arrhythmias are caused by reentrant excitation it can be expected that during cooling the wavelength prolongs. We found that during slow pacing and during pacing at the maximal pacing frequency the wavelength prolonged when the temperature was lowered from 37 °C to 29 °C. A further lowering of the temperature from 29 °C to 23 ° C resulted in a shortening of the longitudinal and transverse wavelength, but compared to 37 °C the wavelength was not changed. During pacing at the maximal pacing rate a decrease of the temperature from 29 °C to 23 °C resulted in a further prolongation of the longitudinal wavelength (+25%) due to a prolongation of the refractory period (193%) which exceeded the slowing of conduction (-50%). The transverse wavelength was slightly shortened (-20%) because the slowing of conduction (-67%) counteracted the prolongation of the refractory period. The prolongation of the longitudinal and transverse wavelength during cooling from 37°C to 29 °C thus can explain the reduced vulnerability to ventricular reentry during hypothermia.

Effects of epinephrine and lidocaine.

Epinephrine administration increases the risk of the initiation of ventricular arrhythmias in humans (Lown and Verrier, 1971). Epinephrine shortens the refractory period (Noble, 1975, Millar et al., 1985) while conduction velocity is not changed (Antoni and Merzweck, 1967). We found that during epinephrine administration the refractory period shortened (-17%), however because of a small nonsignificant increase of the conduction velocity this resulted only in a minimal shortening of the wavelength. The increased risk of the initiation of arrhythmias can not be explained by the small shortening of the wavelength Presumably the increase of the sinus rate during epinephrine administration, or the accelaration of an ectopic pacemaker may account for the increased risk of arrhythmias (Cranefield, 1974, Noble, 1975).

Lidocaine is one of the most frequently used anti-arrhythmic drugs during the acute phase of a myocardial infarction (Lie et al., 1974). It prolongs the refractory period despite a shortening of the action potential duration and depresses conduction velocity (Josephson et al., 1972, Arnsdorf and Wasserstrom, 1986). In atrial myocardium, lidocaine administration results in a slight prolongation of the wavelength but did not prevent the initiation of reentry (Smeets et al.,1986, Rensma et al., 1988). We found that due to the slowing of both longitudinal and transverse conduction velocity, in ventricular myocardium the wavelength actually decreased despite a prolongation of the refractory period. This is in agreement with the results of clinical studies, which demonstrated that lidocaine is ineffective ineffective in terminating ventricular arrhythmias in the chronic phase after myocardial infarction (Horowitz et al., 1978, Josephson, 1986). During acute ischemia the sodium channels appeared to be extremely sensitive to lidocaine (Lazzara et al., 1978, El-Sherif et al., 1977a) As a consequence, conduction in ischemic areas will be depressed whereas conduction in healthy myocardium is less affected. Because of this selective action reentry circuits within the ischemic myocardium might be prevented.

CHAPTER III

ANISOTROPIC CONDUCTION BLOCK OF THE LEFT RABBIT VENTRICLE.

3.3. INTRODUCTION

Local unidirectional conduction block is a key phenomenon in the initiation of reentry arrhythmias. For some decades the occurrence of local conduction block has been attributed to spatial dispersion in refractory period (Alessie et al., 1958, Han and Moe, 1964, Zipes et al., 1974, Allessie et al., 1976, Boineau et al., 1980, Gough et al., 1985), local differences in excitability (Schmitt and Erlanger, 1928, Cranefield et al., 1971, Cranefield and Hoffman, 1971) or nonuniformity in the amount of excitatory current generated by the cell membrane (Woodburry and Crill, 1961, Cranefield et al., 1971). More recently geometrical factors of the cardiac syncitium have been considered as a potential cause of unidirectional conduction block (De la Fuente 1971, Mendez et al., 1969, 1970, Rawling et al. 1984, Van Capelle et al., 1976, Spach et al.,1981, 1982a, 1982b, 1986, 1987). In a series of excellent papers Spach and coworkers (Spach et al., 1981, 1982a, 1982b, 1983, 1986, 1987) have emphasized that not only branching sites or junctions of muscle bundles may form areas with a low safety factor for propagation, but that also anisotropy in passive electrical properties of the myocardium plays an important role in impulse conduction. The importance of anisotropy in the genesis of reentrant arrhythmias is supported by the observation that ventricular tachycardias, developing 3-4 days after myocardial infarction, originate from a thin surviving epicardial muscle layer showing enhanced anisotropic properties (Gardner et al., 1985, Wit et al. 1982, 1987, Dillon et al., 1988). In normal ventricular myocardium conduction velocity is about three times faster parallel to the longitudinal fiber axis than in a transverse direction (Dräper and Mya-Tu, 1959, Clerc 1977, Roberts et al. 1979, Spach et al., 1981, 1982b). This anisotropy in propagation is caused by directional differences in effective axial resistance and membrane capacitance (Spach et al., 1981, 1987). Spach et al. showed that, as a result of the different degree of electrotonic load on the propagating depolarization wave, the amplitude and rate of rise

of the action potential is higher during transverse conduction than during longitudinal propagation (Spach et al., 1981, 1982a, 1982b, 1986, 1987).

However there is still considerable debate about the role of anisotropy in the occurrence of local conduction block. Spach et al. (1981) have argued that because of the lower current load imposed on the depolarization wave during transverse conduction, the margin of safety for transverse propagation is higher than for longitudinal conduction. They postulated that impulses with a decreased stimulating efficacy would preferentially block in a longitudinal direction (Spach et al., 1981). In their experimental studies on the crista terminalis they found that premature impulses were blocked in a longitudinal direction whereas uniform propagation proceeded in a transverse direction. This example of functional "transverse dissociation" which in some cases led to reentry of the crista terminalis was attributed to anisotropic cellular coupling (Spach et al., 1981). On the other hand there are a number of studies showing preferential block in a transverse direction (Schmitt and Erlanger 1928, Anderson et al., 1970, Myerburg et al., 1973, Delmar et al., 1987). In a computer simulation of impulse conduction in a two-dimensional anisotropic sheet of cardiac cells Van Capelle (1983) was unable to produce longitudinal block. However they found that a critically timed premature impulse could be blocked in a transverse direction.

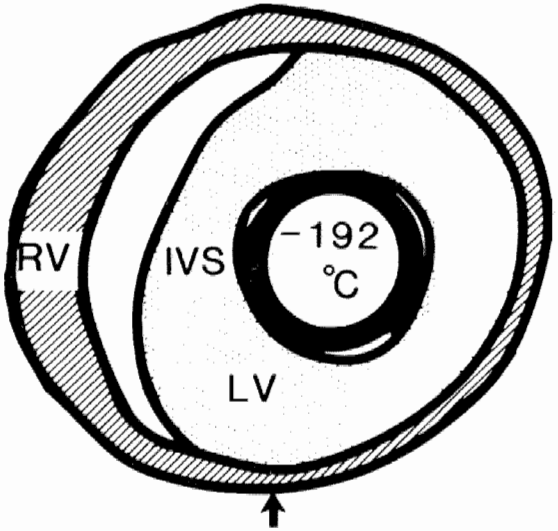
The aim of the present study was to investigate the role of anisotropy in conduction block and reentry in a perfused two-dimensional sheet of uniform anisotropic ventricular myocardium. We found that if the safety factor of conduction was lowered, either by premature stimulation, rapid pacing or elevation of extracellular potassium, conduction block first occurred in a transverse direction. In contrast to its frequent appearance however, transverse block rarely led to reentry, because the tissue distal to the arc of transverse block was activated by fast longitudinal conduction. The rapid activation of the area distal to a transverse block provided insufficient delay for the cells proximal to the block to restore their excitability and reentry was thus often prevented. On the other hand, if (more rarely) a line of longitudinal block occurred, the chance of initiation of a reentrant tachycardia was much higher because of slow transverse conduction distal to the arc of longitudinal block.

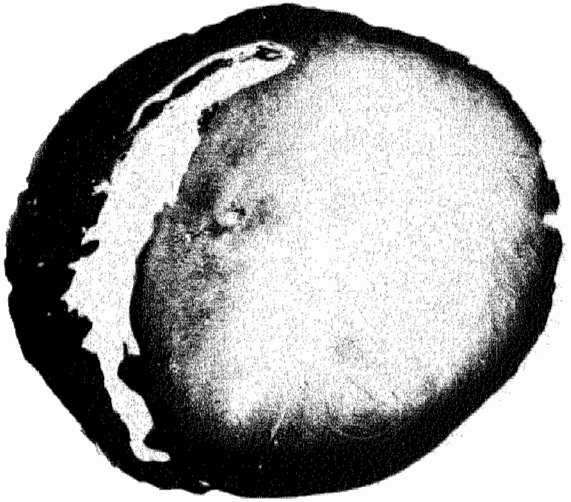
3.2. METHODS

Flemish rabbits (n = 40) of both sexes weighing between 4.5 and 6.5 kg were used in this study. After heparinization (1000 I.U/I.V.) the animals were killed by a cervical dislocation. The thorax was opened by a midsternal incision and the heart was rapidly removed and placed in cold perfusion fluid (10°C). The aorta was cannulated and the heart was connected to a Langendorff perfusion system. The coronary arteries were perfused with a perfusion pressure of 50 mm Hg, resulting in a flow of 35-45 ml/min. The millimolar composition of the perfusion fluid was: NaCl 130; $NaHCO_3$ 20.1; KCl 5.6; $CaCl_2$ 2.2; $MgCl_2$ 0.6; Na_2HPO_4 1.2 and glucose 12. The solution was saturated with a mixture of 95% O_2 and 5% CO_2 pH was 7.35, and temperature was kept at 37°C ± 0.5. In some experiments the potassium concentration of the perfusion fluid was increased by infusion of KCl (0.28 M) into the aorta cannula with an infusion pump (Razel A-99).

To study the anisotropic properties of the epicardium of the left ventricle, the endocardial and intramural layers were destroyed by freezing (see figure 3.1). The Langendorff perfused rabbit heart was immersed in a tissue bath, containing perfusion fluid of 30°C. A cryoprobe was installed in the left ventricular cavity and the coronary circulation was temporarily interrupted. The cryoprobe was then filled with liquid nitrogen (-192°C) and the heart was frozen for 7 minutes. After this period the coronary circulation was restored and the probe removed. In some cases the cryoprobe was also inserted in the right ventricular cavity without interrupting the coronary circulation and without submerging the heart in the tissue bath. As a result of this procedure the endocardial and intramural parts of the free wall of the left ventricle, the total right ventricular wall and the interventricular septum were destroyed. Only a thin left ventricular epicardial layer of about 1 mm thick survived. After freezing coronary flow was 0 to 20 % lower than during control (perfusion pressure 50 mmHg). The heart was allowed to recover for at least 20 minutes before measurements were made. To evaluate the cryoprocedure, at the end of the experiment the heart was perfused with TTC (a buffered 2,3,5 Triphenyl Tetrazolium Chloride solution, Merck). This substance is a specific indicator of dehydrogenase enzymes by yielding a bright red formazan pigment. No staining occurs at sites where the myocardium has lost its dehydrogenase activity. This macroscopical staining method has been shown to correlate well with the (ultra)structural signs of myocardial necrosis as demonstrated with standard histologi-

CRYOPROCEDURE





cal or electronmicroscopical techniques (Nachlas and Shnitka, 1963, Fishbein et al, 1981, Vivaldi et al., 1985).

Recording and stimulation.

For high resolution mapping of epicardial excitation a rectangular (13 x 15 mm) electrode, containing 192 electrodes with an interelectrode distance of 1 mm was used. Programmed electrical stimulation was performed with a programmable constant current stimulator delivering square pulses of 2 ms duration and 2-4 times diastolic threshold. Experimental protocols included pacing at different rates and the application of one to three premature stimuli. Stimuli could be applied to any pair of electrodes of the multi terminal probe. For regular pacing a stimulus strength of two times threshold was used. The intensity of premature stimuli was set at four times threshold. Unipolar electrograms were recorded using the cannula in the aorta as indifferent electrode. The recorded electrograms were fed into 192 individual amplifiers (bandwidth 2 - 400 Hz) and displayed in groups of eigth on two Tektronix 5103N oscilloscopes. The outputs of the amplifiers were multiplexed (sampling rate 2000 Hz), digitised (8 bit) and after Pulse Code Modulation (Kaiser PCM system K1280-00) recorded on tape (Ampex PR 2230). After the experiment the data were analyzed using a PDP 11-73 computer (Digital). An algorithm to detect the intrinsic negative deflection in the electrograms was used to mark local activation times. Isochrone maps were displayed on the computer video display (VT240, Digital). Details of the mapping system enabling simultaneous recording of 192 electrograms have been described elsewhere (Allessie et al, 1984).

Figure 3.1. Left: Schematic representation of the cryoprocedure. A plastic test-tube (diameter 12 mm) was inserted into the cavity of the left ventricle and filled with liquid nitrogen. The epicardium was protected by immersing the heart into a tissue bath of 30°C. As a result the interventricular septum and the endocardial and intramural layers of the free wall of the left ventricle were destroyed. Only a thin epicardial layer of about 1 mm thick survived. Right: TTC staining after the cryoprocedure. The dark epicardial rim of tissue represents surviving myocardium. In this case the right ventricle was not frozen and was also stained by TTC.

Measurement of conduction velocity and refractory period.

Epicardial conduction was analyzed using the rectangular high resolution mapping electrode. The heart was stimulated through a pair of stimulating electrodes in the centre of the probe. One axis of the electrode was positioned parallel to the epicardial fiber direction. This alignment was checked on basis of the highest amplitude and slope of the electrograms recorded along the long fiber axis and by the shortest possible conduction time (Kleber et al, 1986). Conduction velocity was measured both in a longitudinal (V_L) and in a transverse direction (V_T), the degree of anisotropy being expressed by the V_L / V_T ratio. In addition complete epicardial velocity maps were constructed by calculating the vector of impulse propagation from the activation times of four neighbouring electrodes.

Refractory periods were determined at nine different epicardial sites during pacing with a cycle length of 350 or 150 ms. After each 15th regular stimulus a single premature stimulus (4 times diastolic threshold) was applied and the functional refractory period was determined as the shortest attainable interval between the regular and the premature impulse (shortest V1-V2 interval) at a recording electrode close to the site of stimulation.

To correlate the electrophysiological axis of longitudinal conduction with the anatomical fiber direction the position of the mapping electrode was marked by stainless steel pins and the block of tissue was cut out. After fixation in 4% formaldehyde serial sectioning (thickness 10 μm) was performed parallel to the epicardial surface and the sections were stained with Hematoxiline-eosine. Photographs of the sections were used to compare the epicardial fiber axis with the spread of excitation.

3.3. RESULTS

Morphological effects of total endocardial freezing.

To investigate the anisotropic properties of a Langendorff perfused rabbit heart a cryotechnique was used to destroy the endocardial and intramural parts of the left ventricle leaving only a thin layer of epicardial fibers intact. After freezing, the surviving epicardial layer remained adequately perfused through the coronary arteries. Perfu-

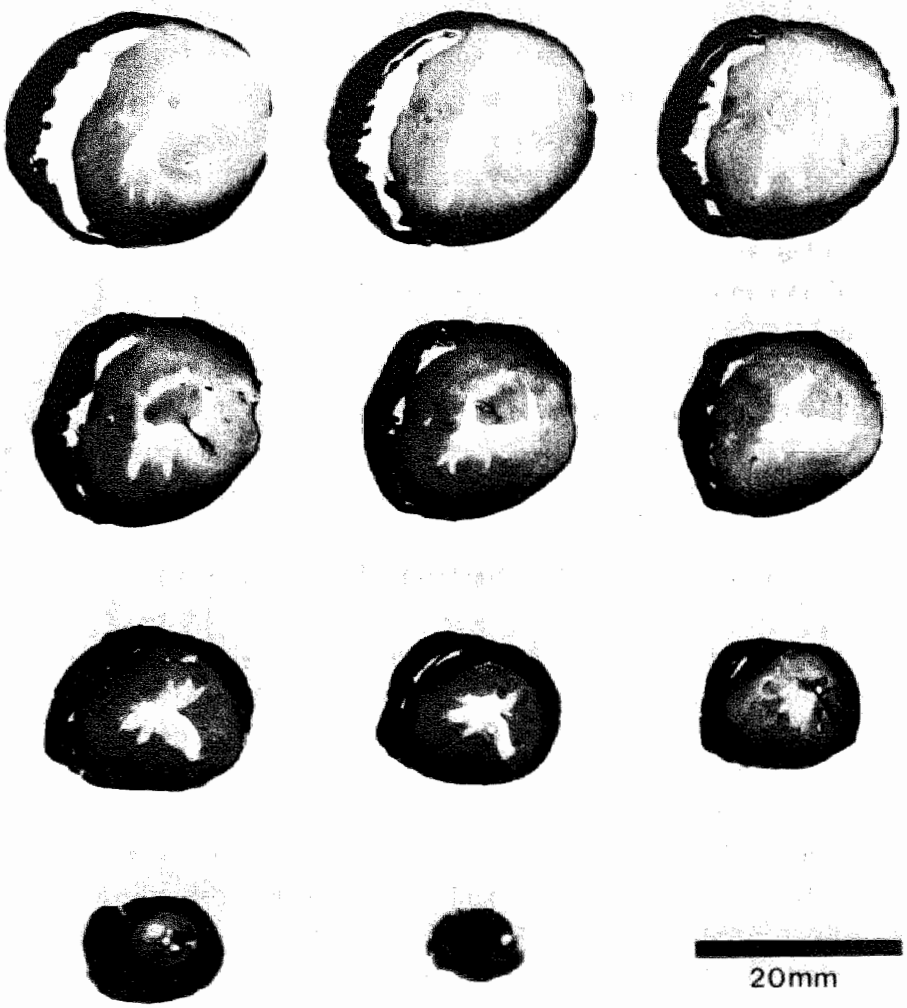


Figure 3.2. A series of 2mm thick slices of rabbit ventricle, cut parallel to the AV-ring and stained with TTC after the cryoprocedure. The darkly stained myocardium represents surviving tissue. Surviving and destroyed myocardium are sharply separated. No surviving cells could be detected in the destroyed myocardium. Despite the fact that the right ventricle was not frozen, the interventricular septum was completely destroyed.

sion with triphenyl tetrazolium chloride (TTC) was used to distinguish between surviving and destroyed tissue. After staining, the ventricles were cut in 2 mm thick sections parallel to the AV ring from base to apex. Figures 3.1 and 3.2 show an example in which the surviving epicardial layer can be recognized by its dark color. In this case only the left ventricular cavity was frozen and therefore in addition to the surviving left epicardial layer, the right ventricle completely consisted of viable myocardium. The transition between surviving and dead tissue was sudden. Histological examination showed no islands of viable tissue in the destructed parts of the myocardium and no dead fibers in the surviving epicardium. The thickness of the surviving epicardial wall, measured at intervals of 2 mm in 11 hearts was 1.0 $\pm$ 0.4 mm (n = 99). The variation in local thickness of the surviving muscle layer was caused by the presence of epicardial fat and major blood vessels. At places where epicardial fat or a coronary artery was present, the surviving rim of the underlying myocardium was thinner. Obviously the interposition of these structures between the epicardium and the tissue bath decreased the protecting effect of the warm surrounding perfusion fluid during freezing.

Electrophysiological evaluation of the surviving epicardial layer.

To investigate the effects of the cryoprocedure, refractory periods were measured at 9 different epicardial places of the free wall of the left ventricle. In figure 3.3 the mean epicardial refractory periods as measured in 10 hearts during regular pacing with a cycle length of 350 ms before and after freezing are shown. In the intact heart local refractory periods varied between 166 and 177 ms (upper numbers) (mean 171 $\pm$ 11 ms). After freezing (lower numbers) the refractory period ranged between 165 and 184 ms (mean 172 $\pm$ 10 ms). At none of the recording sites the endocardial freezing procedure resulted in a statistically significant change in refractory periods. Also the maximal pacing rate (F_{max}) was not affected by the freezing procedure. Before and after freezing the interval of F_{max} was less than 90 ms.

The effects of the cryoprocedure on the epicardial spread of excitation was also evaluated (figure 3.4). The epicardium was stimulated through a pair of electrodes in the center of the mapping electrode. In the upper part of figure 3.4, electrograms recorded along and transverse to the fiber orientation are shown.

Before freezing the electrograms recorded along the longitudinal fiber axis (vertical) exhibited smooth high amplitude biphasic deflections and gradually prolonging

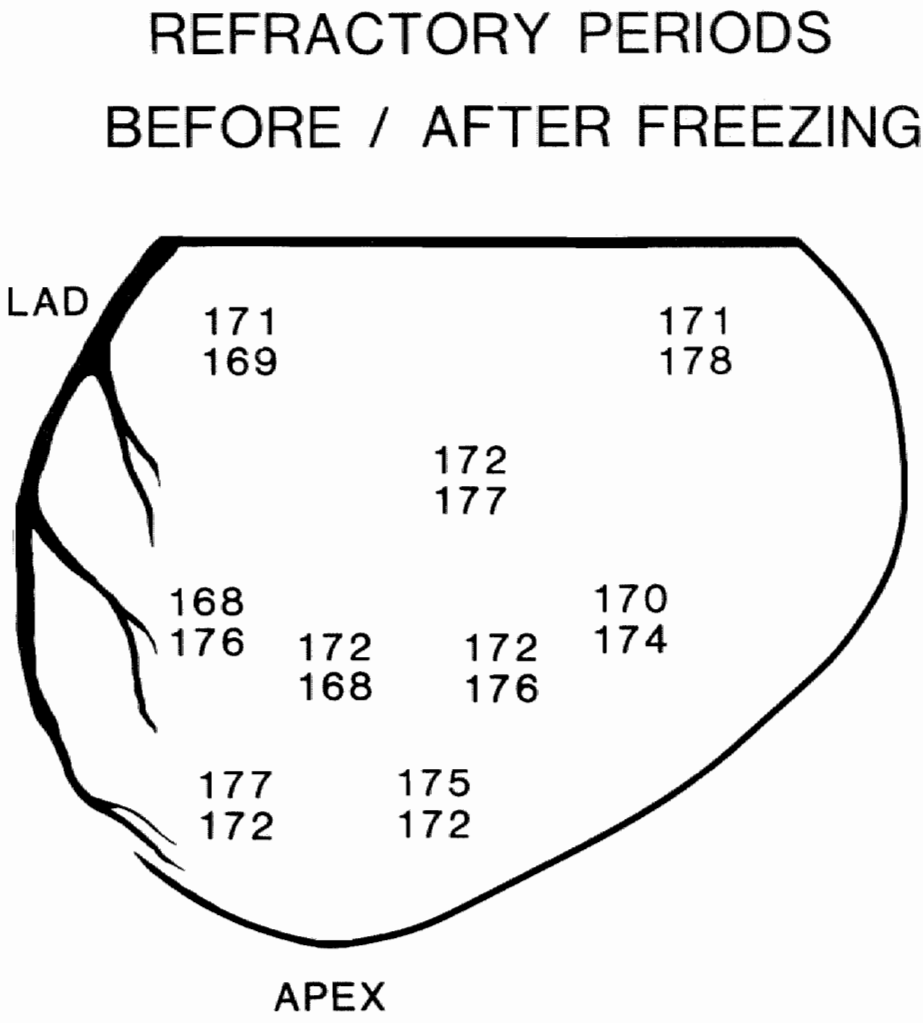
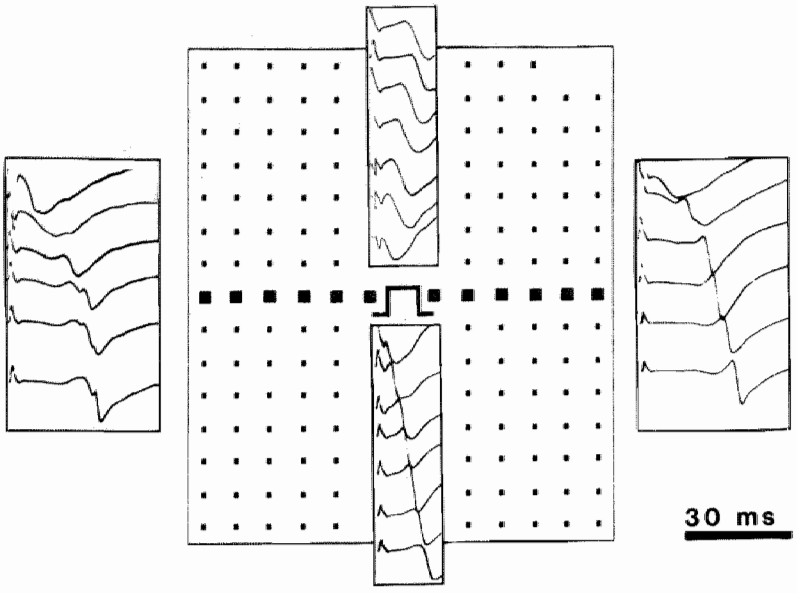
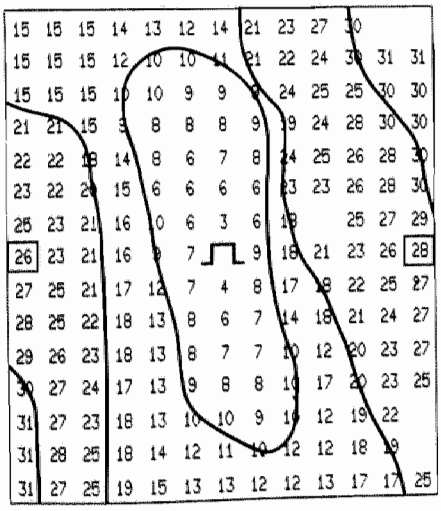


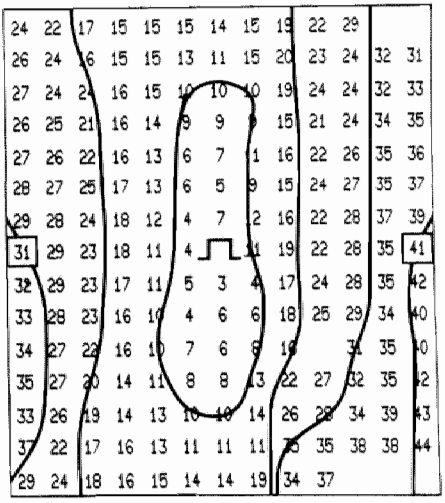
Figure 3.3. Average values of epicardial refractory periods measured in 10 hearts at 9 different epicardial locations before and after freezing. The values measured during control are plotted above the average duration of the refractory period after the cryoprocedure. At none of the epicardial sites the refractory period was altered significantly after freezing.



CONTROL



AFTER FREEZING



activation times. The row of horizontal electrodes orientated perpendicular to the fiber axis showed a different pattern. Electrograms recorded close to the stimulation electrodes were of low amplitude with no or only small R waves, typical for uniform transverse propagation (Spach et al., 1979, 1981a, 1986). Already within a distance of 3 mm from the stimulation site, however, the characteristics of transverse propagation disappeared and electrograms typical for longitudinal conduction were recorded. This transition from transverse to longitudinal electrograms was accompanied by a sudden decrease in interelectrode conduction times. In the lower panels of figure 3.4, epicardial activation is shown before and after freezing. Propagation along the fiber axis was not affected by the cryoprocedure and the activation times of the distal longitudinal electrograms remained unaltered. However in a transverse direction the activation times were clearly prolonged. During control the transverse distal electrodes were activated at 26 and 28 ms compared to 31 and 41 ms after freezing.

To study transverse conduction over a longer distance, the epicardium was stimulated through a pair of electrodes at the periphery of the electrode array. In figure 3.5 activation maps of transverse conduction before and after freezing are given together with maps of the local conduction velocities. Again removal of the subepicardium resulted in a marked prolongation of the transverse conduction time. In this example the time required to activate the area under the mapping electrode was increased from 40 to 83 ms. From the velocity maps at the bottom of figure 3.5 it can be seen that the prolongation of conduction was not caused by a depression of transverse conduction velocity. Instead the longer conduction times were the result of a greater effective distance of slow transverse conduction. In the intact heart stimulation at the peripheral pair of electrodes produced an initial transverse epicardial wavefront which however

Figure 3.4. Epicardial conduction before and after freezing.
In the upper panel electrograms recorded from four rows of electrodes (positioned in a cross) are compared. During longitudinal conduction (vertical axis), high amplitude biphasic deflections were recorded. During transverse conduction (horizontal rows) only the two or three proximal electrograms were typical for transverse conduction. Also the conduction time between electrodes increased suddenly after 2-3 mm from the site of stimulation. In the lower panels the activation maps recorded during pacing with 350 cycle length before and after freezing are shown. The impulse spread in an ellipsoidal pattern with fast conduction parallel to the fiber axis and slow conduction perpendicular to it. Longitudinal conduction was not altered by the cryoprocedure. However, transverse conduction times were markedly prolonged after freezing, the activation times measured at the distal transverse electrodes increasing respectively from 26 and 28 ms before freezing to 31 and 41 ms after freezing.

was interrupted after about 3-4 mm by epicardial breakthrough of a deeper longitudinal wavefront. As a result large areas of the epicardium were activated nearly simultaneously resulting in a sudden increase in the calculated local conduction velocities. True transverse epicardial impulse propagation was assumed when the local conduction velocities were less than 30 cm/s (shaded area). Comparing the velocity maps before and after freezing, it is obvious that the area which is activated by transverse propagation is considerably enlarged by removal of the subepicardial muscle layers.

In figure 3.6 the effects of endocardial freezing on the effective distance of transverse conduction are plotted. In panels A and B a series of 8 electrograms are shown recorded along the transverse axis of the epicardium. In the intact heart (A) epicardial breakthrough is apparent at the third electrogram. After freezing (B) the sudden changes in morphology and interelectrode conduction time had disappeared and transverse conduction proceeded along the whole line of electrodes. In panel C the mean values of interelectrode conduction times along the transverse fiber axis as measured in 12 experiments are plotted as a function of the distance to the site of stimulation. Within 3 mm from the point of stimulation conduction times were not statistically different. However at greater distances the local differences in conduction times became significantly shorter in the control hearts compared to the frozen hearts ($p<.05$). Thus the destruction of the subepicardium by endocardial freezing did not depress the velocity of epicardial impulse propagation nor did it change the ratio between longitudinal and transverse conduction velocities. The major effect was a selective prolongation of the distance over which slow transverse conduction could proceed without being interrupted by epicardial breakthrough. This resulted in a considerable enlargment of the effective area of anisotropy.

Anisotropy and conduction block.

We tested the hypothesis of preferential anisotropic conduction block in three different ways. 1) By gradually increasing the extracellular potassium concentration. 2) By incremental rapid pacing at normal potassium concentrations, and 3) by the induction of multiple premature beats.

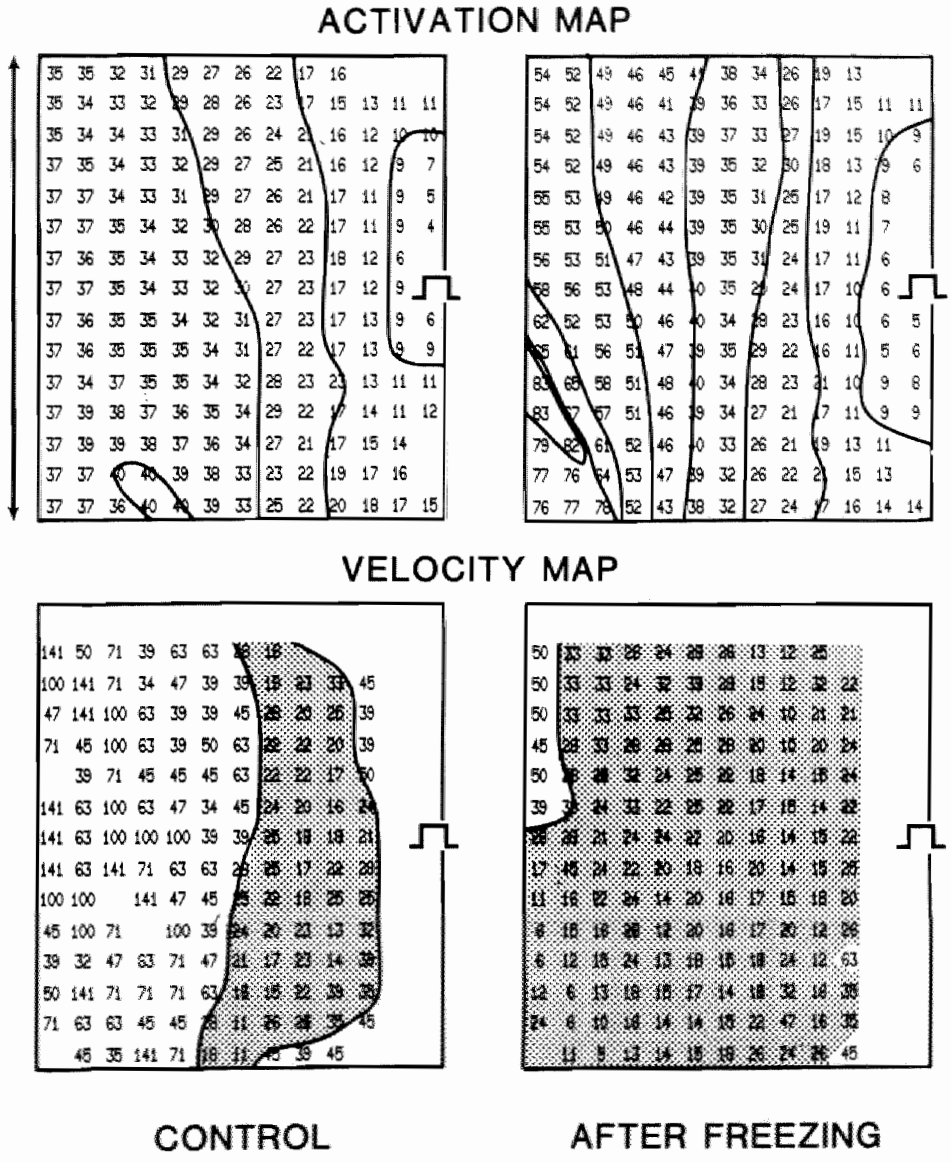
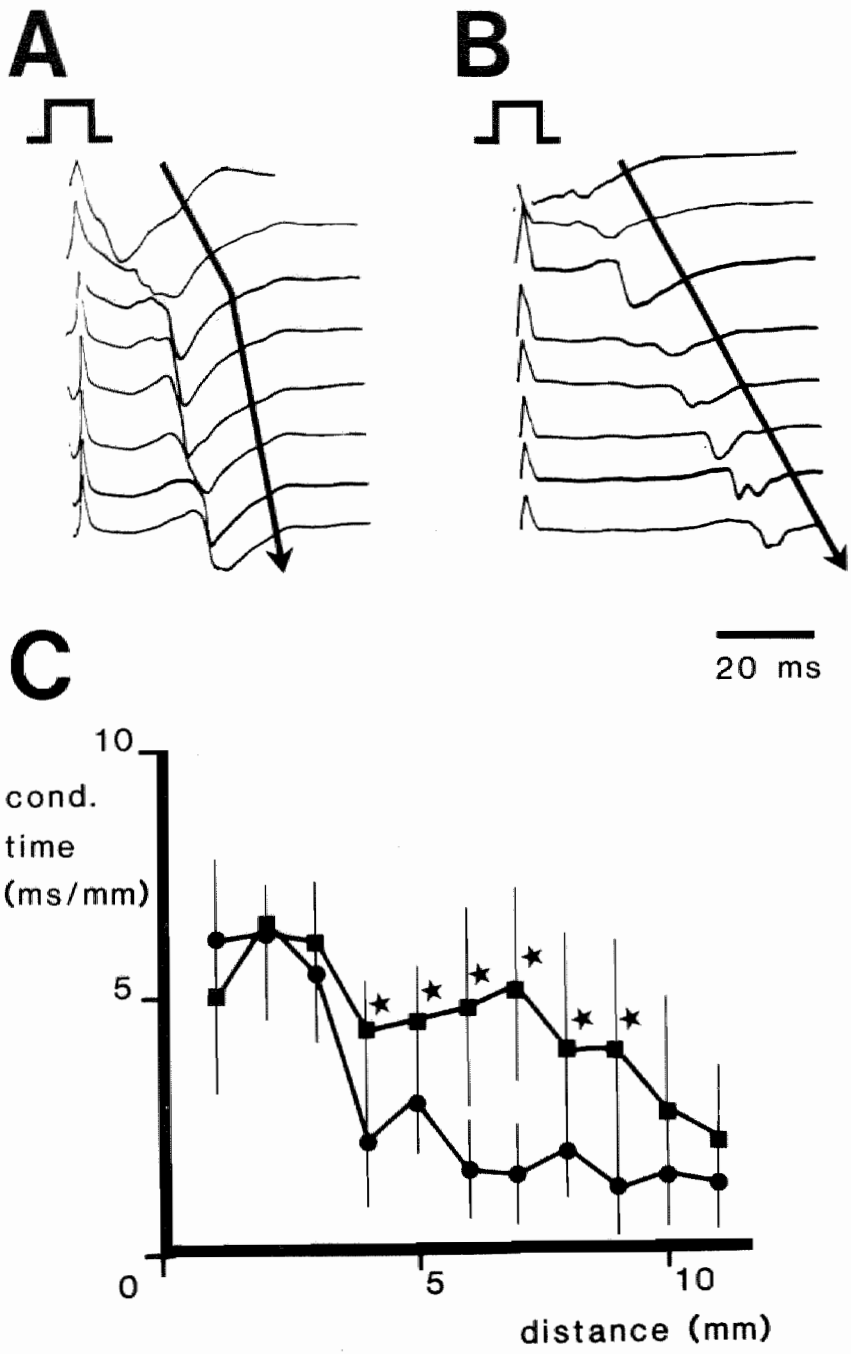


Figure 3.5. Activation maps (top) and velocity maps (bottom) of transverse conduction before and after freezing. The epicardium was paced through a pair of stimulating electrodes at the right border of the mapping electrode. In the velocity maps, the area of transverse conduction with a velocity lower than 35 cm/s is stippled. Before freezing at a distance of less than 4 mm, transverse epicardial conduction was interrupted by epicardial breakthrough of the impulse from deeper layers. After removal of the subepicardial layers by freezing the effective distance of transverse conduction was considerably increased because epicardial breakthrough could no longer occur.


A
B
20 ms
C
10
cond.
time
(ms/mm)
5
0
5
10
distance (mm)

High extracellular potassium

Increasing the extracellular potassium results in a progressive decrease of the membrane resting potential and a depression of the fast sodium inward current. This leads to a gradual diminishment of the safety factor for impulse conduction and a lengthening of the refractory period (Dominquez and Fozzard, 1970, Tsuboi et al., 1985). Because we wanted to exclude the possibility that conduction block could occur on basis of existing spatial dispersion in refractory periods, the ventricles were paced at a very slow rate (cycle length 2000 ms) to ensure complete recovery of excitability in all parts of the epicardium. In figure 3.7 the epicardial propagation during slow pacing from the center of the mapping electrode is shown at three different concentrations of potassium. During control (K^+ = 5.6mM), normal anisotropic conduction was recorded with a V_L:V_T ratio of 3.5 (V_L = 74 cm/s; V_T = 21 cm/s). No signs of impaired conduction or local conduction block were seen. At low heart rates (30/min) impulse conduction proved to be very resistant to increased extracellular potassium. At a concentration of 19.0 mM conduction velocity was still not different from control and local conduction block was not observed neither in a longitudinal nor in a transverse direction. At an extracellular potassium concentration of 24.0 mM impulse propagation became clearly depressed. In a transverse direction crowding of isochrones and lines of conduction block (thick lines) can be seen. The areas retrograde to the block were activated in a longitudinal direction by the impulse turning around the edges of the lines of transverse block. Both the position and the length of the lines of block were not fixed but could change from beat to beat emphasizing the functional nature of the conduction block. In contrast to the frequent occurrence of transverse block, longitudinal block was not observed until the myocardium became totally inexcitable. The electro-

Figure 3.6. Transverse electrograms before and after freezing. Before freezing transverse conduction was recorded by the two or three proximal electrodes. At more remote sites electrogram morphology showed characteristics of longitudinal conduction and inter-electrode conduction times decreased. After freezing the sudden change in interelectrode conduction time had disappeared and transverse electrograms were recorded by all electrodes. In the lower part of the figure the interelectrode conduction times are plotted before (●) and after (■) freezing. At distances more than 3-4 mm away from the site of stimulation significant differences in conduction time were found.

(* = $p < 0.05$)

EFFECTS OF POTASSIUM

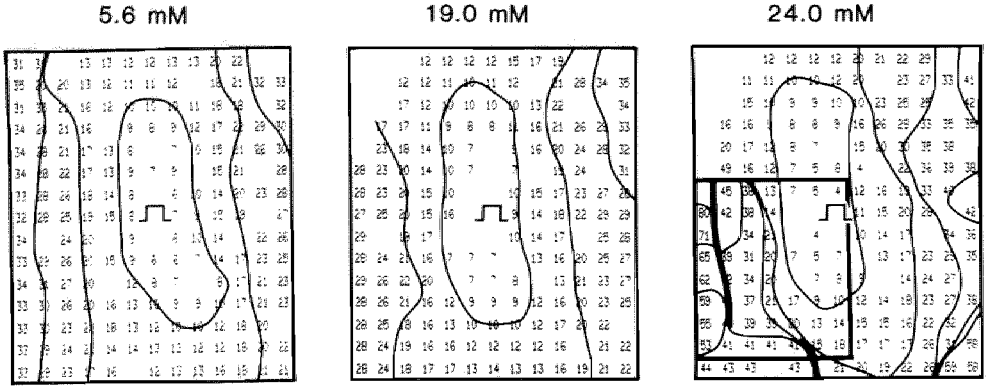
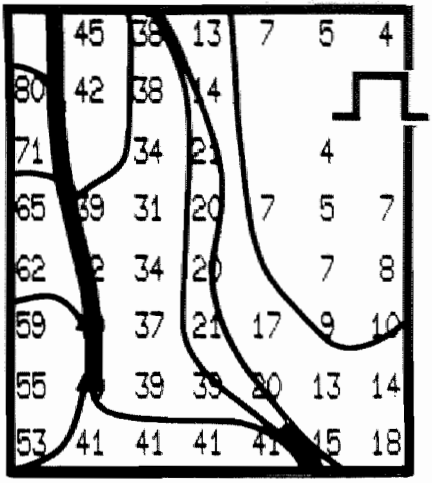
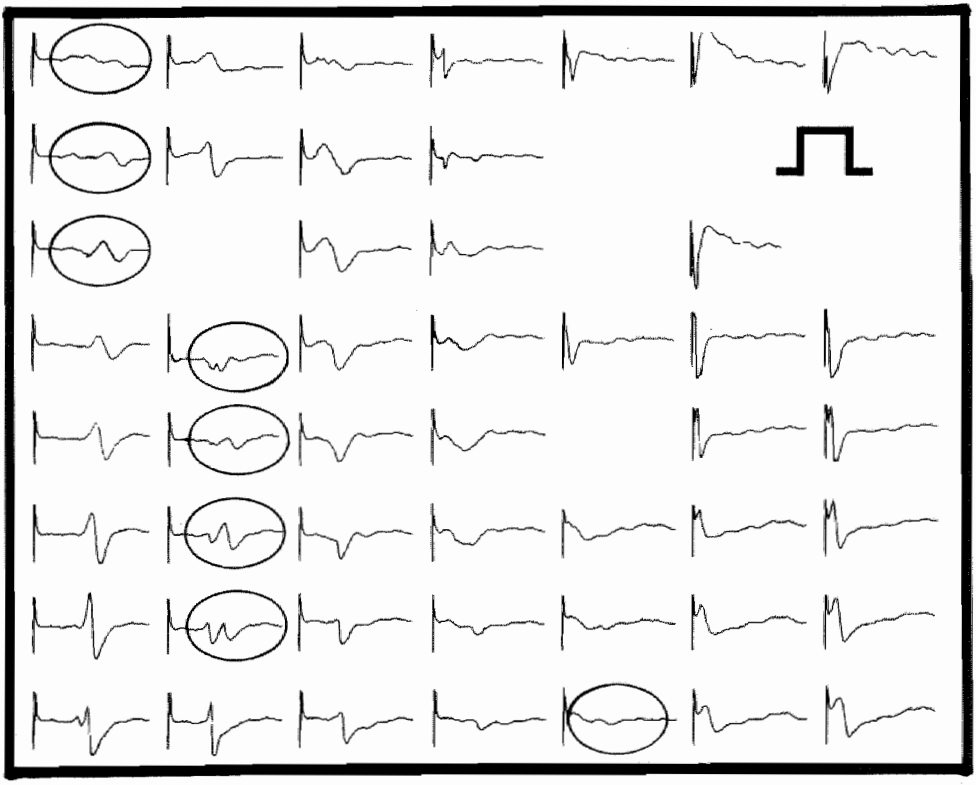


Figure 3.7. Activation maps at three different extracellular potassium concentrations (5.6, 19.0, and 24.0 mM). The heart was paced at a regular interval of 2000 ms from the center of the mapping electrode. The rectangular area delineated in the map recorded at 24.0 mM potassium, shows an arc of transverse conduction block and is plotted in figure 3.8 together with the corresponding electrograms.

grams associated with the phenomenon of transverse block are plotted in figure 3.8. High amplitude electrograms are recorded at sites where the impulse propagates longitudinally to the fiber orientation (vertical). Low amplitude signals correlate with transverse propagation. Electrograms recorded from the transverse line of block consisted of two distinct deflections, the first caused by the approaching transverse conducting wavefront, and the second due to electrotonic current flow from the retrograde longitudinal impulse. The double component electrograms did not show multiple low amplitude fragmented deflections which are seen during slow transverse conduction in nonuniform anisotropic tissue (Spach et al., 1982a, 1986).

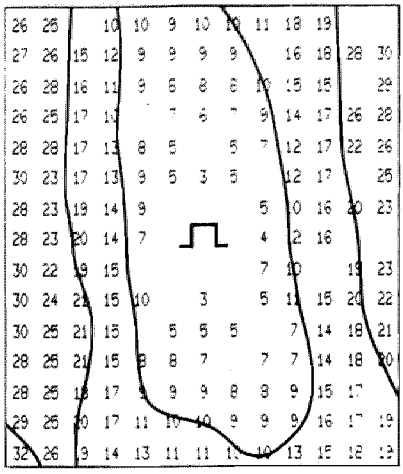
Figure 3.8. Demonstration of transverse conduction block by high extracellular potassium (24.0 mM). The area corresponds with the rectangular area indicated on the map in figure 7. In the lower part of the figure the electrograms recorded at the respective sites of the map are given. The electrograms start at the moment of the stimulus. A time window of 100 ms is displayed. The encircled electrograms were recorded at the line of transverse conduction block.


45 38 13 7 5 4
80 42 38 14
71 34 21 4
65 59 31 20 7 5 7
62 2 34 20 7 8
59 37 21 17 9 10
55 39 35 20 13 14
53 41 41 41 41 15 18

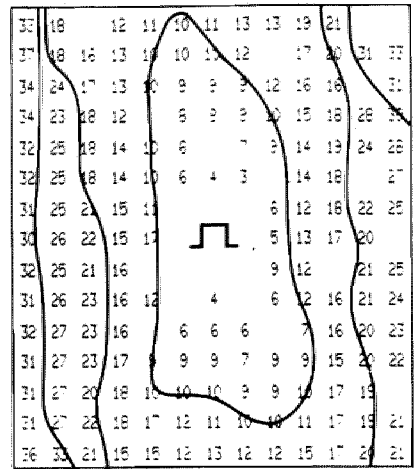


EFFECTS OF RATE

350 ms

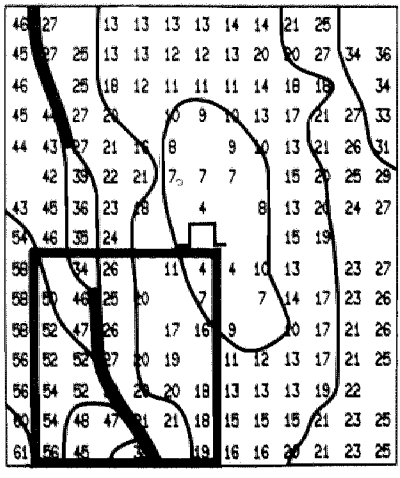


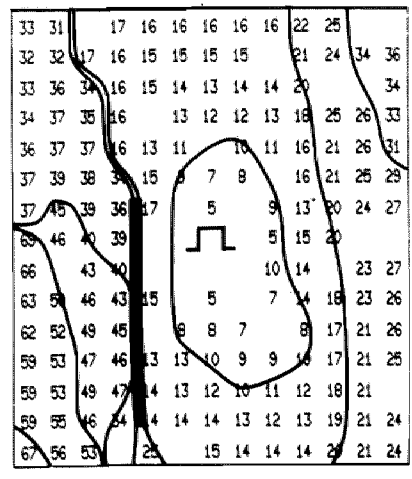
200 ms



110 ms

ALTERNANS





Rapid pacing

The effects of incremental rapid pacing are shown in figure 3.9. Activation maps are given during pacing with a cycle length of 350, 200 and 110 ms. During pacing with an interval of 350 ms the spread of excitation was regular with fast conduction (65 cm/s) parallel to the fiber orientation and slower conduction (20 cm/s) in a transverse direction (V_L/V_T ratio 3.25). No regions of conduction block or impaired conduction were found. During pacing with a cycle length of 200 ms this pattern of activation was unaltered and longitudinal and transverse conduction velocity were only slightly decreased. When the pacing rate was increased to 110 ms an electrical alternans developed. In the lower part of figure 3.9 activation maps of the alternating beats are given. The maps show that the electrical alternans is based on an alternation in pattern of activation. Especially in a transverse direction major differences in conduction between two successive impulses were seen. During one beat two lines of transverse conduction block were present in the left part of the map. In the next beat the two areas of block had disappeared and were replaced by a single longer line of block close to the stimulus site. This pattern of alternating transverse conduction block was stable and repeated itself as long as rapid pacing was continued. The electrograms recorded from the area of rate dependent transverse conduction block are plotted in figure 3.10. The signals recorded at the line of block exhibited double component electrograms. At those sites where the two components are widely separated a clear isoelectric segment was present between the two deflections.

Premature beats

The role of anisotropy on conduction of premature beats is shown in figure 3.11. During regular pacing (S1) two closely coupled premature stimuli (S2 and S3) were

Figure 3.9. The effects of rapid pacing on anisotropic conduction. Top: Activation maps during pacing with a cycle length of 350 and 200 ms. Bottom: Two consecutive activation maps during pacing at a maximal rate (110 ms cycle length). As shown by the electrogram at the top of these maps an electrical alternans existed at this high pacing rate. The delineated area of conduction block in the left lower panel is shown in figure 10 with the corresponding electrograms.

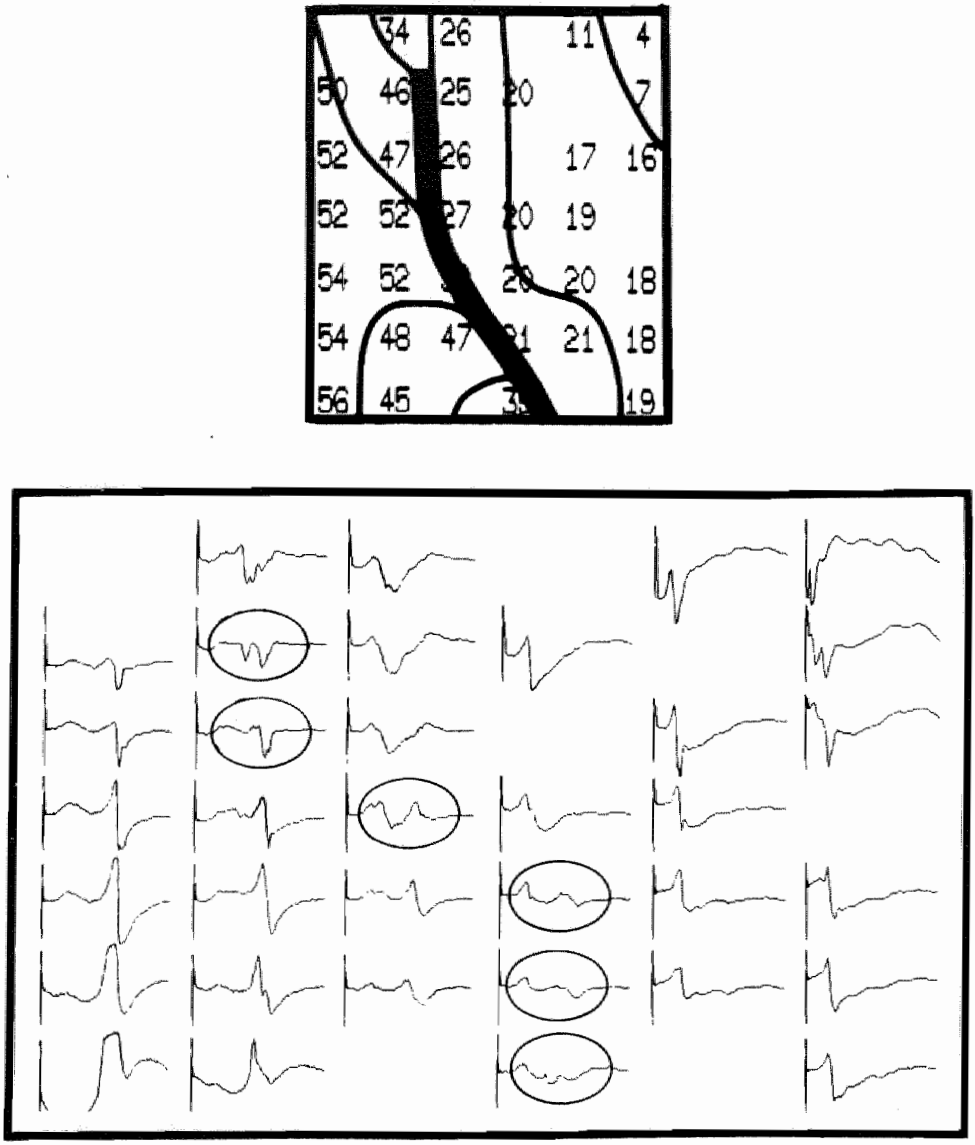


Figure 3.10. Electrograms from an area of transverse conduction block induced by rapid pacing. An electrogram window of 100 ms is displayed starting at the moment of the stimulus. The isochrone map corresponds with the rectangular area indicated on the lower left panel of figure 9. The encircled electrograms were recorded from the line of transverse conduction block.

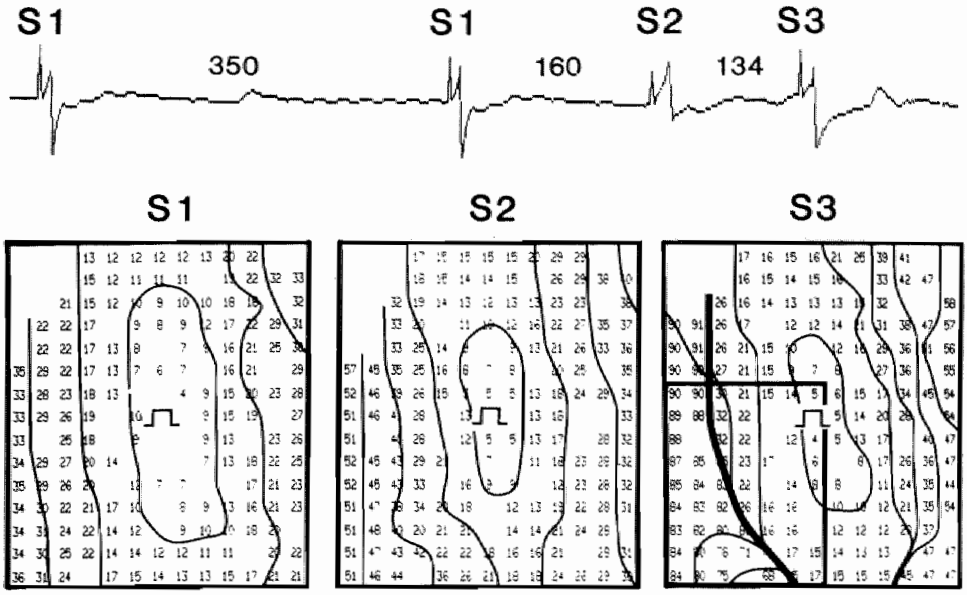
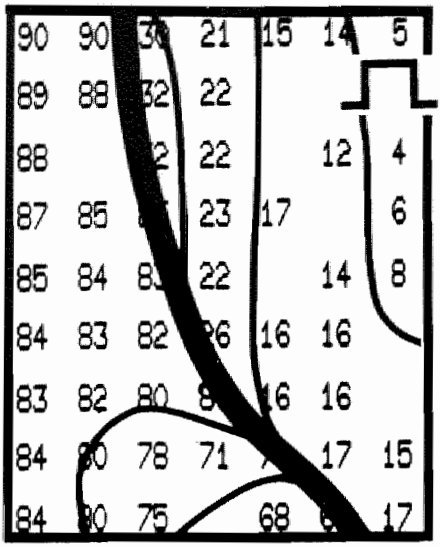
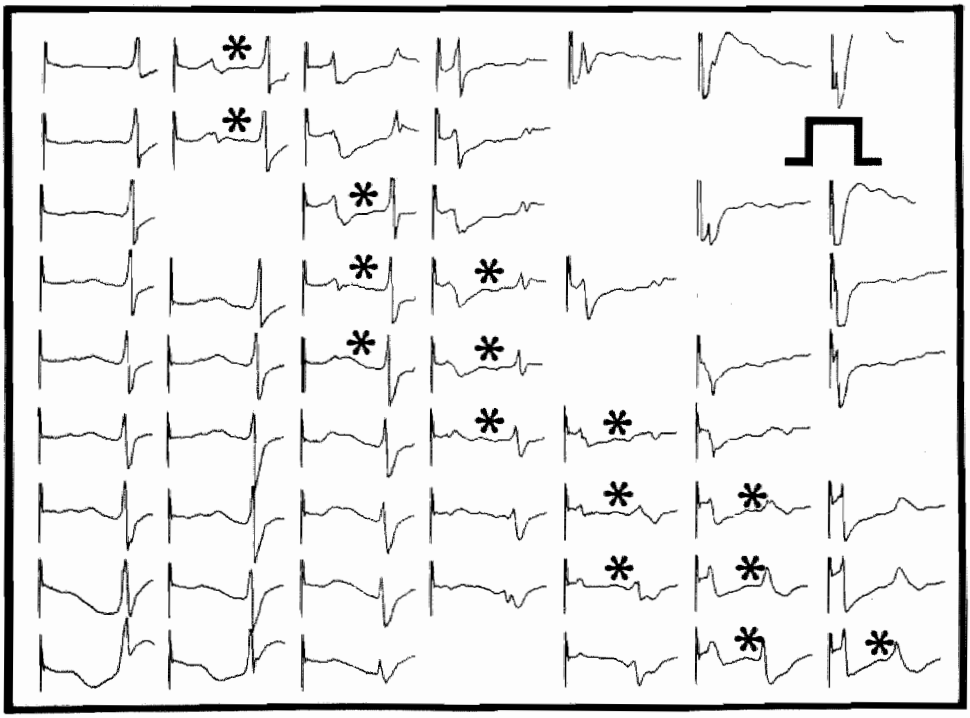


Figure 3.11. The effects of multiple premature beats on anisotropic conduction during regular pacing (S1-S1 = 350 ms). The hearts were paced from the centre of the mapping electrode. Panel S1 gives the activation map during regular pacing. The maps designated by S2 and S3 give the spread of activation during two closely coupled premature beats (S1-S2 = 160ms; S2-S3 = 134ms). During the second premature impulse (S3) a long line of transverse conduction block developed. The myocardium retrograde to the line of block was activated by a longitudinal fast conducting wavefront. The electrograms recorded from the area of block are shown in figure 12.


90 90 30 21 15 14 5
89 88 32 22
88 2 22 12 4
87 85 23 17 6
85 84 22 14 8
84 83 82 16 16
83 82 80 16 16
84 78 71 17 15
84 75 68 17



given. Compared to the regular rhythm, the first premature beat was conducted more slowly both in longitudinal and transverse direction but propagation was still uniform. During the second premature beat marked differences in propagation along both axes became apparent. In the longitudinal direction conduction remained uniform and was not different from the first premature beat. However in a transverse direction conduction became more depressed resulting in further slowing of conduction and the appearance of an arc of transverse conduction block. The myocardium retrograde to the line of transverse block was activated by a longitudinal wavefront pivoting around the lower edge of the line of block. Thus premature impulses showed markedly increased anisotropy because propagation along the transverse fiber axis was more affected than longitudinal conduction. The electrograms recorded from the line of block are plotted in figure 3.12. Proximal to the line of block all electrograms exhibited the characteristics of transverse conduction, e.g. low amplitude deflections with no or small initial R-waves. Again the signals recorded from the line of block (indicated by asterisks) showed double component electrograms. Due to the delay in activation of the area retrograde to the line of block, the two components of the electrograms were widely separated. This makes it easier to see the absence of fragmented electrical activity between the two components of the electrograms. The presence of an isoelectrical segment between the two wavefronts is good evidence that true transverse conduction block of the premature impulse was present.

Anisotropy and reentry.

In the intact isolated rabbit ventricle rapid pacing can induce ventricular fibrillation but ventricular tachycardia was never observed. However after endocardial freezing of the 4/5 part of the left ventricular wall, the opposite was true. Now fibrillation could no longer be induced. Instead the application of one or more premature stimuli or a short period of rapid pacing could initiate episodes of sustained ventricular tachycardia. The cycle length of these tachycardias in the surviving left ventricular epicardium

Figure 3.12. Transverse conduction block and related electrograms during premature activation. The signals start at the moment of the premature stimulus and cover a time window of 100 ms. The electrograms marked with an asterisk were recorded at the line of block. Same experiment as figure 11. See text for further description.

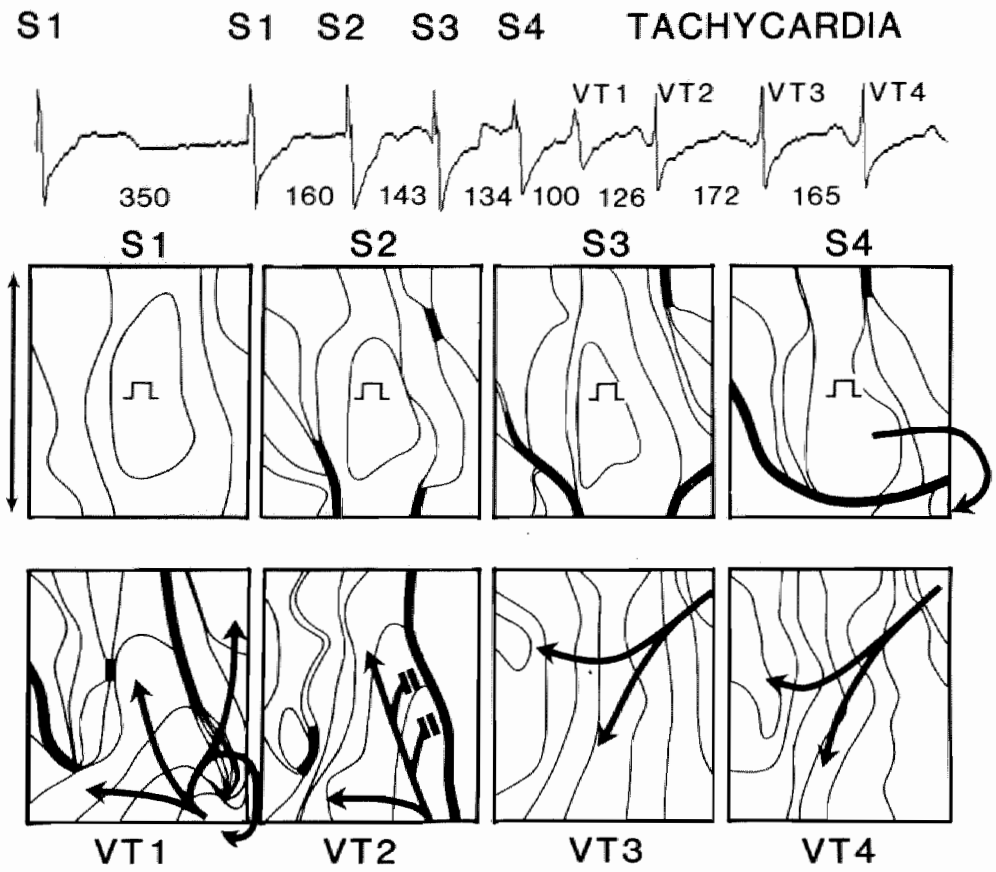


Figure 3.13. Top: Initiation of ventricular tachycardia by three closely coupled premature stimuli (S1-S4). The intervals between the successive beats are indicated in ms.
Bottom: Isochrone maps during regular pacing (S1), premature stimuli (S2-S4) and the first 4 beats of the ensuing ventricular tachycardia (VT1-VT4). The epicardium was paced through a pair of stimulating electrodes in the center of the mapping electrode. The vertical arrow to the left of the S1 map indicates the epicardial fiber orientation; its length corresponds to a distance of 14 mm. During the first and second premature beats (S2 and S3) arcs of local transverse conduction block can be recognized. These transverse blocks did not lead to reentry. During the third premature impulse (S4), block also occurred in a longitudinal direction. Fusion of the lines of transverse and longitudinal block resulted in a long L-shaped arc of block. Ventricular tachycardia was initiated by reentry around this functional arc of block. During the first beats of the tachycardia the morphology of the electrograms and the isochrone maps varied from beat to beat, caused by changes in size and position of the reentrant pathways. During sustained tachycardia, in this case the circuit was located outside the mapping electrode. See text for further discussion.

ranged from 110 to 165 ms (mean 130 ± 11 ms). Once initiated they could last for many hours without any change in cycle length or electrogram morphology.

Figure 3.13 shows the pattern of activation around the site of stimulation during the initiation of ventricular tachycardia by three consecutive premature stimuli. In this case local transverse conduction block occurred already during the first premature impulse (S2). Also during the second premature beat (S3) lines of transverse block can be distinguished. During the third premature impulse (S4) also longitudinal block occurred, resulting in a long L-shaped arc of block. The myocardium retrograde to this long line of block was activated after a long delay by an impulse turning clockwise around the right end of the line of block. In the first map of the tachycardia (VT1), it can be seen that the tissue proximal to the longitudinal line of block was reexcited by this turning impulse, resulting in an extra fast conducting wavefront propagating upwards parallel to the fiber direction. During this first reentrant beat, several regions of transverse conduction block were found. In the right lower corner an area of very slow transverse conduction (less than 5 cm/s) was present, giving rise to a single small reentrant circuit. During the second beat of the tachycardia (VT2), the spread of excitation changed completely. Except for a small line of block in the left part of the map, in the right part a long line of transverse conduction block extended from the lower to the upper border of the mapping electrode. The area of slow transverse conduction present during the first beat of the tachycardia now also participated in the line of block and reentry could no longer be found under the mapping electrode. However the tachycardia did not stop and as can be seen in the third and fourth map of the tachycardia (VT3 and VT4), the area under the electrode was now activated repeatedly by a broad wavefront propagating from the upper right to the lower left corner of the electrode. During the initiation of ventricular tachycardia the cycle length and electrogram morphology of the first three to four beats often change. Obviously before the tachycardia becomes sustained and monomorphic, the circulating impulse moves around (in this case away from the mapping area) before it finds a stable location somewhere else in the epicardium.

3.4. DISCUSSION

The last decade it has become clear that anisotropic properties of the myocardium play an important role in impulse conduction and the genesis of cardiac arrhythmias (El-Sherif et al., 1977b, 1981, 1985, Fenoglio et al., 1983, Gardner et al., 1985, Wit et al., 1982, 1987, Dillon et al., 1988). In the intact ventricle the myocardial fibers are orientated parallel to each other, with the longitudinal fiber axis gradually changing from epi- to endocardium over a total angle of about 120 degrees (Streeter 1979). By this rotation of the fiber axis the effect of anisotropy on impulse propagation is mitigated because slow transverse conduction is bypassed by impulses arriving earlier from the neighboring layers. This explains that in the ventricular wall, despite the presence of considerable anisotropy, the overall myocardial conduction velocity is governed by longitudinal propagation (Durrer and van Dam, 1957, Durrer et al., 1970). It also means that the local loss of myocardium resulting in survival of thin layers of cells, will lead to an increased influence of anisotropy on impulse conduction.

Ventricular anisotropic conduction has been studied in isolated sheets of myocardium excised from the right or left ventricle and in papillary muscle preparations (Clerc, 1976, Spach et al., 1982b, 1986, Tsuboi et al., 1985, Kadish et al., 1986, Delmar et al., 1987). To maintain stable conduction, such experiments had to be carried out at reduced temperatures (25- 30^{o}C) or within a limited range of pacing frequencies. Under normothermic conditions the conduction velocity of in vitro preparations is strongly rate dependent and conduction block occurs at a lower pacing frequency than in the perfused heart (Spach et al., 1982b, Tsuboi et al., 1985, Delmar et al., 1987). To avoid some of the problems related to superfused preparations, we used an endocardial freezing technique to create a thin layer of perfused epicardium (1.0 $\pm$ 0.4 mm). The electrophysiological properties like refractory period, conduction velocity and maximal pacing rate were not different from control. The removal of the subepicardium however had an important effect on the distance over which transverse epicardial conduction could proceed. In the intact ventricle transverse conduction was interrupted within 3-4 mm from the site of stimulation by epicardial breakthrough of an impulse from deeper layers. In the two-dimensional preparation slow transverse conduction continued undisturbed over a distance of more than 8 mm.

Anisotropic conduction and conduction block.

The theoretical and experimental studies of Spach and co-workers (1981, 1982b, 1983, 1986, 1987) have raised evidence that the continuous cable theory developed by Hodgkin and Huxley for impulse transmission in nerve (1952) does not give an adequate description of impulse propagation in cardiac tissue. In the heart discontinuities of effective axial resistivity exist at several size levels and together with possible differences in membrane capacity this may be responsible for local differences in safety of propagation and a variety of cardiac conduction disturbances (Sommer and Dolber, 1979, Sommer, 1983, Joyner, 1982, Diaz et al., 1983, Spach et al., 1981, 1982a, 1986, 1987). On the basis of the observed directional differences in propagation velocity and the inverse relationship between V_{max} and conduction velocity Spach et al. (1981) predicted that: "Propagation can become decremental and stop in one direction while it continues as uniform propagation in the other direction. In fact, uniform propagation ceases and becomes decremental first in the direction having the highest velocity, as the membrane activity is depressed. The low-velocity wavefront continues to propagate as the membrane is depressed still further. Thus, the "safety factor" is lower when the velocity is high and vice versa . Experimental support for this concept was recently obtained by Tsuboi et al. (1985) who found that in thin sections of canine right ventricular muscle superfused by a solution containing 10 mM potassium, longitudinal propagation failed while conduction in a transverse direction continued. On the other hand, Van Capelle (1983) and Rudy et al. (1987) were unable to confirm the concept of preferential longitudinal conduction block. In computer simulations of impulse propagation in discrete cellular structures they found that V_{max} displayed a bi-phasic behavior as a function of increasing axial resistance. At a moderate increase of the coupling resistance V_{max} first increased, to drop sharply when the coupling resistance was further raised. The sudden decrease in upstroke velocity of the action potential at high coupling resistances was explained by inactivation of the regenerative inward current during the foot of the slowly propagating action potential. Computer simulations of the propagation of early premature beats actually showed preferential block in a transverse direction and as stated by Van Capelle (1983): "Do not support the idea that transverse "slow" propagation is less susceptible to conduction block than is longitudinal "fast" propagation". In a recent study Delmar et al. (1987) investigated the effects of increasing intercellular resistance in isolated sheep epicardial muscle by the cellu-

lar uncoupler heptanol. Under these conditions they found that in all cases block occurred more promptly for transverse than for longitudinal propagation. Transverse conduction block was accompanied by "foot potentials" resulting in partially inactivating the sodium channels (Van Capelle, 1983, Delmar 1987, Rudy et al., 1987).

Our mapping studies of perfused rabbit epicardium showed that when the stimulating efficacy of the depolarization wave is decreased, either by premature beats, rapid pacing or elevating extracellular potassium, transverse conduction block always occurs before propagation in a longitudinal direction fails. This is in agreement with the experimental studies of Delmar et al. (1987) and the computer simulations of Van Capelle (1983) and Rudy et al. (1987). Obviously, when conduction becomes critical, the higher V_{max} of transverse conduction does not compensate for the higher transverse axial resistance and a smaller amount of electrotonic current is delivered to the cells downstream of the crest of the depolarization wave.

Anisotropic conduction and reentrant arrhythmias.

Anisotropic conduction may play an important role in the pathogenesis of atrial and ventricular arrhythmias (El-Sherif et al., 1981, Mehra et al., 1985, Wit et al., 1982, 1985, Dillon et al., 1987). The greater incidence of atrial fibrillation with age may be caused by a redistribution of collagen and progressive electrical uncoupling of myocardial fibers, the resulting higher degree of nonuniform anisotropy enabling the occurrence of unidirectional conduction block and providing small areas of slow conduction for intramyocardial micro-reentry (Spach et al., 1986). Since atrial fibrillation is based on the presence of multiple wandering wavelets the fibrillatory process will tend to be more stable if more reentering impulses are present (Moe, 1962, Allessie et al., 1985). Spear et al. (1983a, 1983b) showed that after myocardial infarction the degree of anisotropy is enhanced by collageneous septa dividing the myocardial fibers. In the studies of Wit et al. (1982, 1987) and El-Sherif et al. (1983) on ventricular tachycardia 3-4 days after myocardial infarction, reentrant circuits were found in the thin epicardial layer overlying the infarcted area. The conduction properties of this layer of cells exhibited greatly enhanced (nonuniform) anisotropy (Spear et al., 1983a, 1983b, Richards et al., 1984, Fenoglio et al., 1983, Gardner et al., 1985, Dillon et al., 1988). In our experiments the effects of enhanced effective anisotropy were studied by the remo-

val of deeper layers without the additional morphological and electrophysiological changes caused by myocardial infarction (Spear et al., 1983a, 1983b).

One of the interesting findings was that, after removal of the subepicardial layers sustained episodes of ventricular tachycardia could be induced by rapid pacing or induction of one or more premature beats. Epicardial mapping during rapid pacing and premature beats frequently showed long lines of transverse block which however did not lead to reentry. The apparent benign nature of transverse block in relation to initiation of reentry is explained by the rapid activation of the area distal to the line of block by longitudinal propagation. On the other hand, if longitudinal block occured, the region behind the block was activated by slow transverse conduction providing more time for the fibers proximal to the block to restore their excitability. For reentry to occur, the minimal length of a line of longitudinal block can be much shorter than a line of transverse block. The higher the degree of anisotropy, the smaller the line of longitudinal block necessary for initiation of intramyocardial reentry. Enhanced anisotropy thus amplifies the role of existing spatial inhomogeneities in conduction properties. Small areas of depressed conduction, which under normal circumstances will not lead to arrhythmias, may then start to act as pivoting points for reentry. Also during sustained tachycardia anisotropy may play an important role because during its roundtrip the impulse has to propagate twice perpendicular to the fiber orientation. The resulting slowing of conduction during transverse propagation will make the dimensions of the circuit smaller than they would be in isotropic myocardium. The presence of areas of nonuniform anisotropy (Spach et al., 1982a, 1986) might further diminish the size of intramyocardial circuits and influence the properties of sustained ventricular tachycardia.

CHAPTER IV

ANISOTROPIC REENTRY AS A MECHANISM OF SUSTAINED VENTRICULAR TACHYCARDIA

4.1. INTRODUCTION

Ventricular tachycardia is a frequent and life threatening event in patients recovering from myocardial infarction (Wellens et al., 1972, 1974, 1975a, Josephson et al., 1978, 1986). Normal and abnormal automaticity, triggered activity, and reentry may cause ventricular tachycardia, however from extensive epicardial and endocardial mapping studies performed in these patients, reentry emerged to be the most likely mechanism of sustained ventricular tachycardia (Josephson et al., 1978, 1986, De Bakker et al., 1983, Josephson and Wellens, 1984). Prerequisites for the initiation of reentry are local unidirectional conduction block and slow conduction. Once trapped in a circuituous pathway, the impulse can circulate around a gross anatomical obstacle (Mines, 1914, Lewis et al., Rosenblueth and Garcia Ramos, 1947, Frame et al., 1986) or around a functionally determined area of conduction block (Allessie et al.,1973, 1977, 1984, 1987, Wit et al.,1987, Dillon et al., 1988). Conduction block and slow conduction were long thought to be caused by local differences in excitability (Mines et al.,1914, Schmitt and Erlanger, 1928, Han and Moe, 1964, Merx et al., 1977, Allessie et al.,1976). Later studies have suggested that it also may be caused by geometrical factors influencing the safety factor for conduction (Mendez et al., 1969, 1970, De la Fuente, 1971, Spach et., 1981, 1982a, 1982b, 1987). Results of experimental studies using canine models, either with permanent or transient occlusion of the coronary artery, suggest that surviving myocardial cell layers in the border zone of the infarct are involved in the pathogenesis of sustained tachycardia (El Sherif et al.,1977, 1981, 1985, Mehra et al., 1983, Wit et al, 1982, 1987, Dillon et al., 1988). Although anatomical barriers sometimes may be present, Wit et al. (1987) and Dillon et al.(1988) demonstrated that sustained ventricular tachycardia initiated in the canine left ventricle 3-4 days after

myocardial infarction, was caused by reentrant excitation involving a functionally determined area of conduction block. They showed that the anisotropic properties of the epicardium overlying the infarct area were greatly enhanced. This enhanced nonuniform anisotropy facilitated the initiation of local conduction block and reentry (Wit et al., 1987, Dillon et al., 1988). However in post infarct ventricles ischemic alterations of the borderzone cells may also contribute to the initiation of reentry by enhancing the inhomogeneity in excitability and the stimulating efficacy of the cells (Spear et al., 1983a, 1983b, Richards et al., 1984, Janse, 1986, Wit et al., 1987, Dillon et al., 1988). In the previous chapter we demonstrated that when the effective area of anisotropy of the left ventricle was enhanced by removal of the intramural and endocardial layers, local conduction block and reentry could be initiated by either premature stimulation or rapid pacing. To study the effects of enhanced tissue anisotropy on the characteristics and mechanisms of ventricular tachycardia the two dimensional model of anisotropy presented in the previous chapter was used. The results of this study demonstrated that sustained ventricular tachycardia could be initiated in the epicardial layer. Reentry was the underlying mechanism of these tachycardias. The impulse circulated mostly around a line of conduction block orientated parallel to the epicardial fiber direction. This line of conduction block was functionally determined. However despite the functional nature of "anisotropic" reentry an excitable gap was present. Due to the presence of an excitable gap anisotropic reentry was stable and longlasting.

4.2. METHODS

Recording and stimulation

Detailed activation maps were reconstructed during sustained tachycardia, using either a high resolution rectangular mapping electrode (192 leads, silver wires diameter 0.3mm, interelectrode distance 1 mm) or a "spoon" shaped electrode covering the apex and the free wall of the left ventricle, containing 384 leads (silver wires diameter 0.3 mm, interelectrode distance 2 mm).

The "spoon" electrode covered the entire free wall of the left ventricle and the apex. The left boundary of the mapping electrode was at the anterior interventricular groove

and the right boundary at the posterior interventricular groove. Because the apex is a more or less circular structure the lower six rows of the spoon electrode are continuous. The activation maps recorded with the "spoon" electrode are displayed into a two-dimensional matrix. The interelectrode distance was 2 mm when all 384 electrograms were recorded. It was also possible to record from half of the number of electrodes but then the interelectrode distance along the X-axis was 4 mm and along the Y-axis 2 mm.

To study the properties of the central line of conduction block during anisotropic reentry in detail a high density mapping electrode was used, containing two rows of 48 electrodes (silver wires, diameter 0.05 mm) with a spatial resolution of 0.185 mm. After localization of the circuit this electrode was moved across the line of conduction block.

Programmed electrical stimulation was performed with a programmable constant current stimulator delivering square pulses of 2 msec duration and 2-4 times diastolic threshold. Stimuli could be applied to any pair of electrodes of the mapping electrode. The inducibility of ventricular arrhythmias was tested before and after freezing by programmed ventricular pacing. The pacing protocol consisted of incremental pacing upto a maximal pacing frequency and the application of single or multiple shortly coupled premature stimuli during regular pacing with a cycle length of 350 ms.

Unipolar electrograms were recorded using the stainless steel cannula in the aorta as an indifferent electrode. The recorded electrograms were fed into 192 individual amplifiers (bandwidth 2 - 400 Hz) and displayed in groups of eigth on two Textronix 5103N oscilloscopes. The outputs of the amplifiers were multiplexed and digitised by three multiplexers, each of which handled 64 signals (Kaiser PCM system K1280-00). Each signal was sampled with a frequency of 2000 Hz and recorded on tape (Ampex PR 2230). After the experiment, data analysis was performed using a PDP 11-73 computer (Digital). An algorithm to detect the intrinsic negative deflection in the electrograms was used to mark local activation times. On basis of the local activation times isochronic maps were displayed on the computer video display (VT240, Digital). Details of the recording system enabling simultaneous measurement of 192 electrodes have been described elsewhere (Allessie et al, 1984).

4.3. RESULTS

Characteristics of sustained ventricular tachycardia.

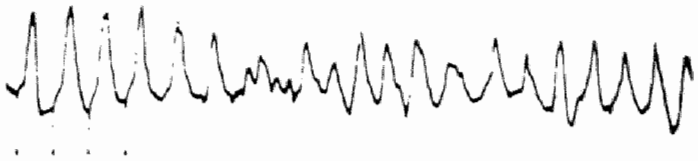
The inducibility of ventricular arrhythmias was tested both before and after freezing by programmed electrical stimulation (figure 4.1). The pacing protocol consisted of incremental pacing and multiple shortly coupled premature stimuli applied during regular pacing (350 ms cycle length). In the intact ventricles, fibrillation or ventricular tachycardia could not be initiated by premature stimuli (figure 4.1 A). Only after prolonged periods of pacing at a maximal pacing rate (90-110 ms pacing interval) ventricular fibrillation was induced (figure 4.1 B).

After the intramural and endocardial layers were destroyed by freezing, sustained ventricular tachycardia could be intiated, in 30 out of 35 preparations, either by the induction of single or multiple premature beats or by incremental pacing (figure 4.1 C, D). These tachycardias were very stable and longlasting (more than several hours in different hearts). The cycle length ranged from 105 to 160 ms (mean 130 ms $\pm$11 ms). Except for the initial 2-4 beats the cycle length and the electrogram morphology showed only small variations in time. However an electrical alternans, both in the cycle length and shape of the recorded electrograms was found during most of the tachycardias. These rhythmic variations were related to the cycle length of the tachycardia. Almost no alternans was recorded when the cycle length was relatively long, while during tachycardia with a high intrinsic rate the alternans was more marked, resulting in a maximal beat to beat variation of 10-15 ms (figure 4.1 C, D). After freezing ventricular fibrillation could not be initiated anymore in any of the preparations, not even by prolonged periods of rapid pacing (90-110 ms cycle length). Functional reentrant excitation is considered to play a major role during atrial or ventricular fibrillation (Moe et al., 1962, Allessie et al., 1985). Because functional reentry tends to terminate spontaneously (Allessie et al. (1977, 1985) a minimal number of simultaneously wandering

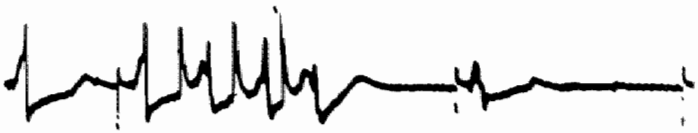
Figure 4.1. Initiation of ventricular arrhythmias in the intact heart and the two dimensional epicardial layer produced by freezing. In the intact ventricle no arrhythmias could be initiated by up to three shortly coupled premature beats. Only prolonged periods of rapid pacing eventually leads to initiation of ventricular fibrillation (A-B). After freezing it was not possible anymore to initiate ventricular fibrillation. Instead sustained ventricular tachycardia could now be initiated either by multiple shortly coupled premature stimuli or rapid pacing (C-D).

BEFORE FREEZING

A. RAPID PACING

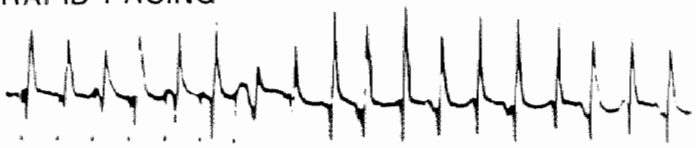


B. PREMATURE BEATS

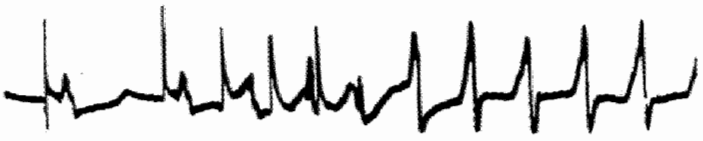


AFTER FREEZING

C. RAPID PACING



D. PREMATURE BEATS



100 ms

wavelets is required for the maintainance of the fibrillatory process (Allessie et al., 1985, Rensma et al., 1988). When the average number decreases the chance of the synchronous inactivation of the wavelets increases and fibrillation will be terminated. The minimal number of wavelets simultaneously wandering through the myocardium depends on the tissue mass. The reduction of the tissue mass after freezing of the intramural and endocardial layers of the left ventricle might explain the fact that fibrillation could not be initiated anymore.

The excitable gap.

It is well known that during sustained ventricular tachycardia, initiated in the post-myocardial infarction period in patients and in canine hearts, an excitable gap is present. During sustained tachycardia in the two dimensional layer a short but clear excitable gap was present. By comparing the cycle length during sustained tachycardia (105 to 160 ms) with the functional refractory periods measured during regular pacing at the same cycle length, the duration of the excitable gap can be estimated to be 10 to 40 ms. The relation between the measured functional refractory period and the cycle length of the tachycardia is given in figure 4.2. During pacing at a cycle length of 100 ms the refractory period was 94 $\pm$ 3 ms which lengthened to about 109 ms $\pm$ 9 ms during pacing at a cycle length of 150 ms. Due to the presence of an excitable gap it was possible to capture the myocardium during tachycardia with single or multiple premature stimuli, and to entrain or terminate the tachycardia by overdrive pacing. In figure 4.3 A it is shown that a single premature stimulus (coupling interval 100 ms) applied during sustained tachycardia with a cycle length of 120 ms, captured the myocardium. The tachycardia was not terminated and resumed its intrinsic rate immediately after the premature beat. In figure 4.3 B an example of entrainment is shown. During a sustained tachycardia with a cycle length of 140 ms the ventricles were paced with a gradually shortened cycle length. As can be seen, during overdrive pacing, each stimulus is followed by a respons indicating entrainment of the tachycardia. When at a pacing interval of 130 ms, pacing was suddenly interrupted, the rate immediately declined to the intrinsic rate of the tachycardia. Eventually, when the pacing frequency was further increased upto 100 ms the tachycardia was terminated (figure 4.3 C).

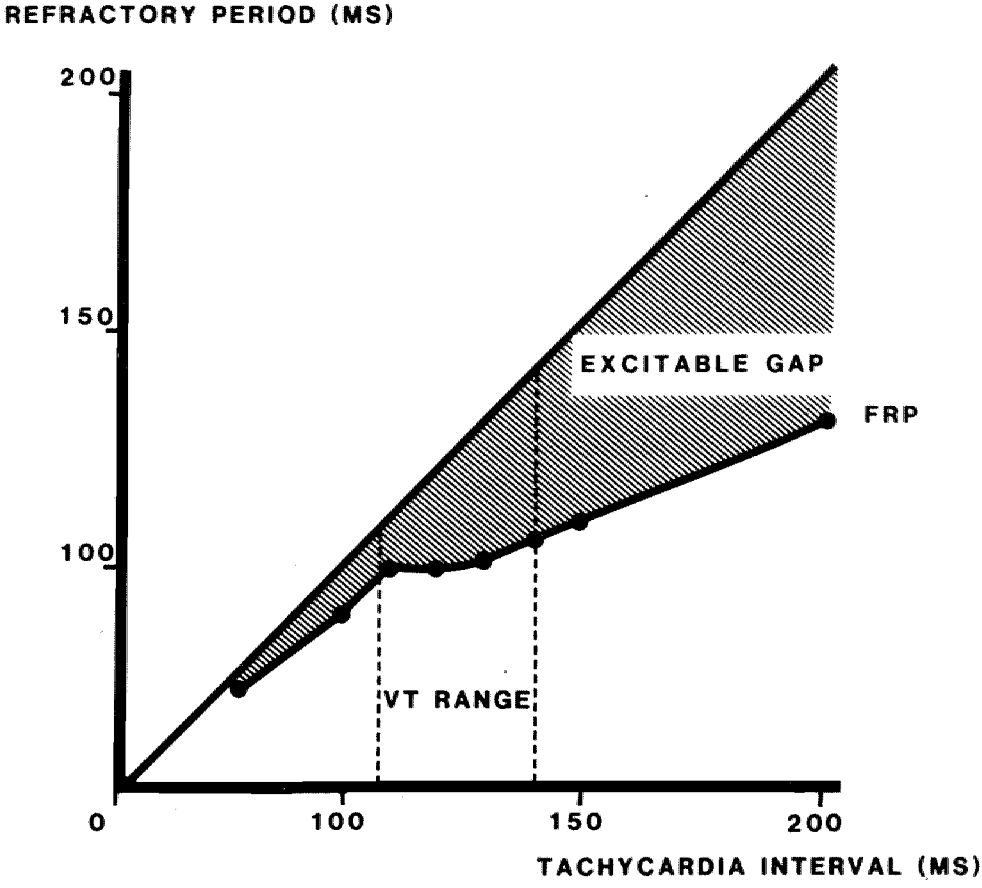


Figure 4.2. The relation between the functional refractory period and the cycle length during tachycardia. During regular pacing at a cycle length of 100 ms the refractory period was 94 ms gradually lengthening to about 110 ms during pacing at a cycle length of 150 ms. The estimated excitable gap during tachycardia ranged from 10 to 40 ms depending on the cycle length of the tachycardia.

Mapping of sustained ventricular tachycardia.

During sustained ventricular tachycardia different patterns of epicardial activation were recorded. Reentrant excitation due to a circulating impulse around the cavities of the ventricles was occasionally found but will not be discussed here. This type of tachycardia was extinguished by freezing of the right ventricle, thereby interrupting the reentrant pathway.

Epicardial reentrant excitation in a single loop was found in 28 out of 30 preparations. In the remaining two preparations a figure of eight type of reentrant excitation was recorded during tachycardia (figure 4.5 D).

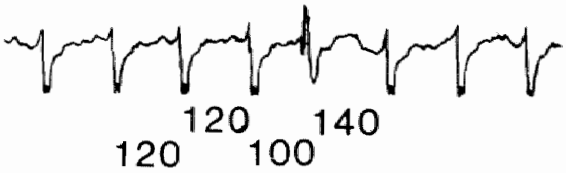
The activation map shown in figure 4.4 was recorded during a tachycardia based on a single reentrant circuit. The cycle length of this tachycardia was 140 ms. The isochrones were drawn at 10 ms time steps and only the central circuit area is displayed. The impulse circulated in a clockwise manner around a long line of conduction block (thick line). The line of block is orientated parallel to the epicardial fiber direction. Due to the anisotropy of the ventricular myocardium the conduction velocity varied around the circuit depending on the direction of impulse propagation. During longitudinal impulse propagation, conduction velocity varied between 50 and 60 cm/s. At the pivoting points, during transverse impulse propagation, conduction slowed down to less than 20 cm/s. The electrograms recorded around the line of block are given in the lower part of figure 4.4. The pattern of activation was regular and each of the recorded electrograms exhibited only minor variations in cycle length and morphology.

In figure 4.5, four activation maps are shown recorded during sustained tachycardia in different hearts.

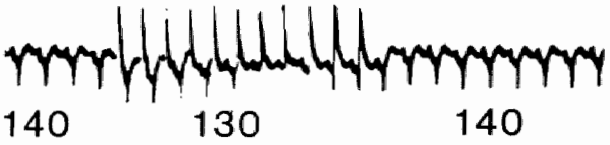
The tachycardia shown in panel A was caused by a single reentrant loop (cycle length 120 ms) circulating in a counter clockwise manner around a long line of transverse conduction block. The impulse conducted rapidly along both longitudinal limbs of the reentrant circuit, while conduction was slower when the impulse propagated around the pivoting points in a transverse direction relative to the fiber orientation. The remaining part of the left ventricle was activated regularly and no other areas of conduction block were found.

Panel B shows the activation map during another tachycardia initiated in another experiment. Again a line of transverse conduction block was present around which the impulse circulated in a regular counter clockwise manner,

A SINGLE STIMULUS



B ENTRAINMENT



C TERMINATION

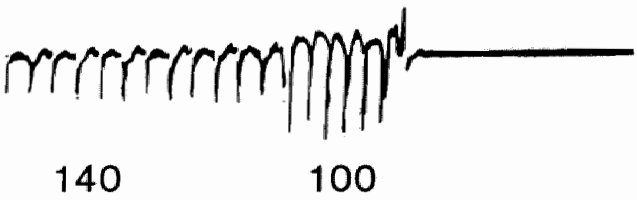
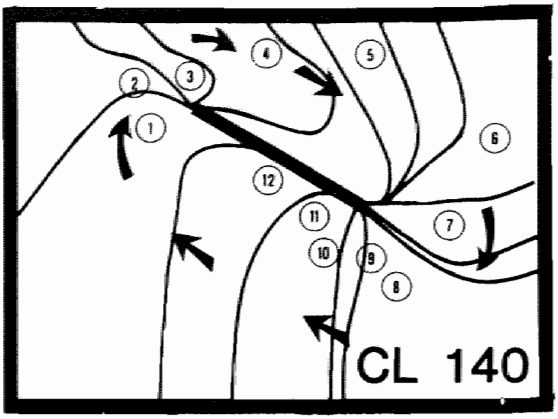


Figure 4.3. Due to the presence of an excitable gap it was possible to capture the heart during sustained tachycardia (cycle length 120 ms) with a single stimulus (coupling interval 100 ms). The tachycardia was not terminated and resumed its intrinsic rate after a compensatory pauze (panel A). Entrainment of sustained tachycardia (panel B). During a sustained tachycardia with a cycle length of 140 ms the cycle length was increased to 130 ms by rapid pacing. After the cessation of pacing the rate immediately decreased again to resume the intrinsic rate of the tachycardia. Eventually, when the pacing interval was further increased to 100 ms the tachycardia was terminated (panel C).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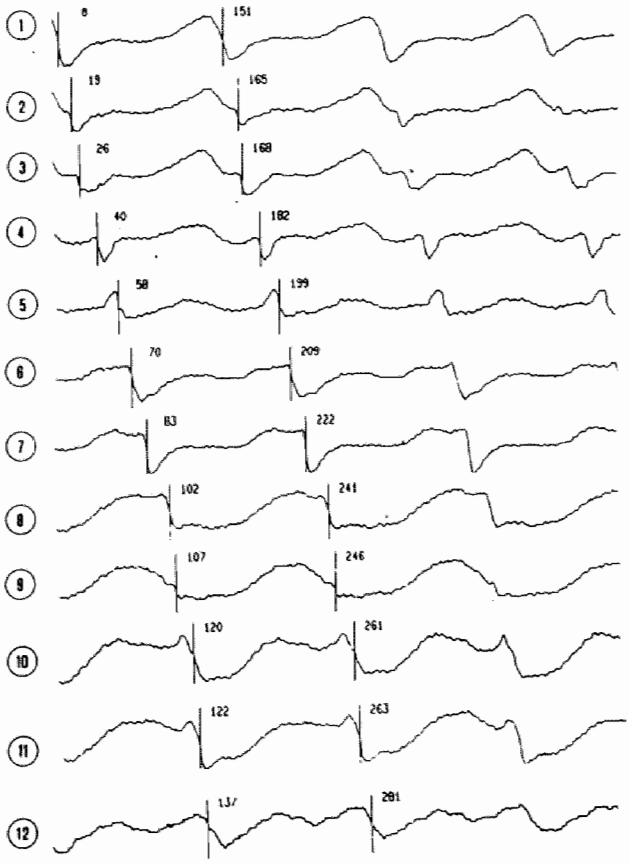
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102
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107
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120
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122
263
12
137
281

with a cycle length of 155 ms. Conduction velocity varied depending on the direction of propagation. Slow conduction was found during transverse conduction when the impulse pivoted around the line of block while fast conduction was found during longitudinal conduction, parallel to the line of block.

The tachycardia shown in panel C was also caused by reentrant excitation. Again only one reentrant circuit (cycle length 120 ms) was present, this time circulating in a clockwise direction around a short line of conduction block. This line of conduction block consisted of one segment orientated parallel to the epicardial fiber direction and another segment orientated perpendicular to the fiber axis. Due to the presence of longitudinal block (orientated perpendicular to the fiber direction), the impulse was forced to propagate in a transverse direction over a considerable distance (reflected by the crowding of isochrones). A second line of conduction block was present in the upper right part of the activation map. This conduction block did not participate in the reentrant circuit.

A figure of eight like reentrant excitation pattern is shown in panel D. The cycle length of the tachycardia was 125 ms. Two lines of conduction block were present, one line of block being orientated more or less perpendicular to the fiber direction (upper left) while the second line was orientated parallel to the fiber direction (lower centre). The impulse conducted around the upper line of block in a clockwise manner. A counter clockwise activation was found around the lower line of block. A narrow common pathway was present between both lines of block through which the impulse conducted slowly.

In figure 4.6 the differences in conduction velocity during ventricular tachycardia are illustrated. Again a single reentrant loop was present, circulating in a clockwise manner around a line of transverse conduction block. Due to the striking anisotropy of the ventricular myocardium conduction velocity varied around the circuit (panel B). During longitudinal conduction, the impulse propagated with a velocity of 52 to 62

Figure 4.4. Electrograms recorded around the line of conduction block. One reentrant circuit was present. The cycle length of this tachycardia was 140 ms. The isochrones were drawn at 10 ms time steps and only the central circuit area is displayed. The electrograms demonstrated regular activation and only minor variations of cycle length and of the electrogram morphology were recorded.

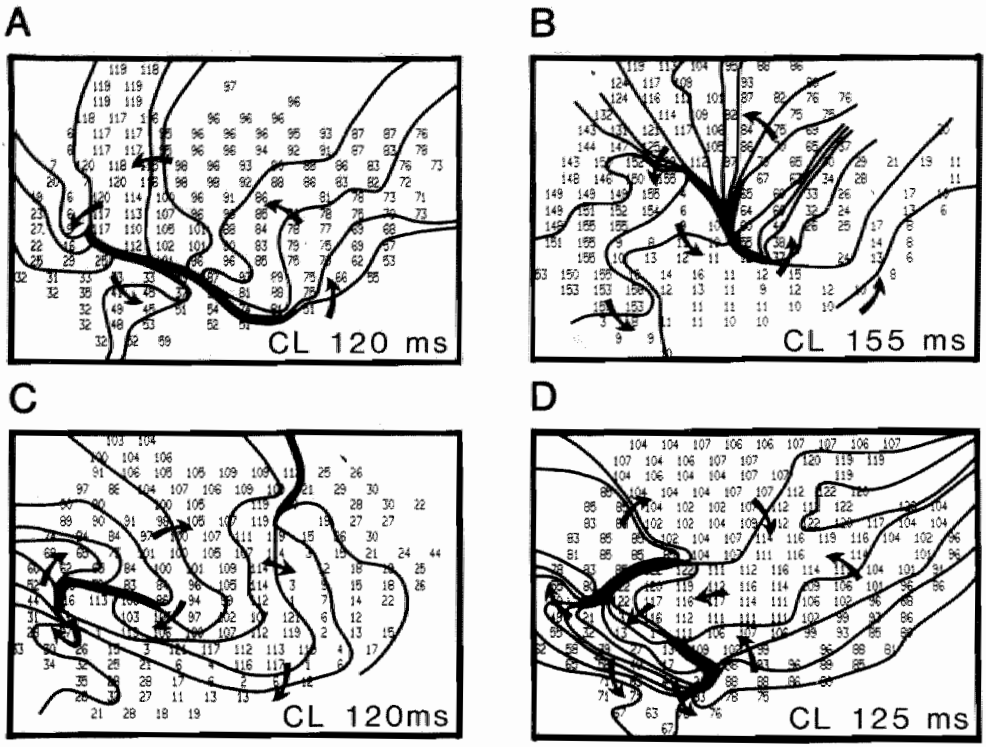


Figure 4.5. Mapping of sustained ventricular tachycardia. Four activation maps, recorded during sustained tachycardia in different hearts. See for explanation text.

cm/s. When the impulse pivoted around the line of block, transverse conduction occurred at a velocity of 14 to 16 cm/s .

The line of central conduction block.

The activation maps given in figure 4.4, 4.5 and 4.6 demonstrate that sustained tachycardia initiated in the normal epicardium of the rabbit ventricle was caused by reentrant excitation. In 75 % of the experiments the impulse circulated around a line of block orientated parallel to the epicardial fiber direction (figure 4.7 A). The length of these lines of conduction block was 20 ±5mm and the mean cycle length of the tachy-

cardias was 129 ±20 ms. A different orientation of the line of block was found in 25 % of the experiments (figure 4.7 B). Sometimes a L-shaped line of block (figure 4.5 C) consisting of a longitudinal and a transverse part was present, while during some tachycardias the orientation of the line of block was found to be related to the orientation of an epicardial blood vessel. The length of these lines of block was 19 6mm and the mean cycle length of the tachycardias was 123 ± 4ms.

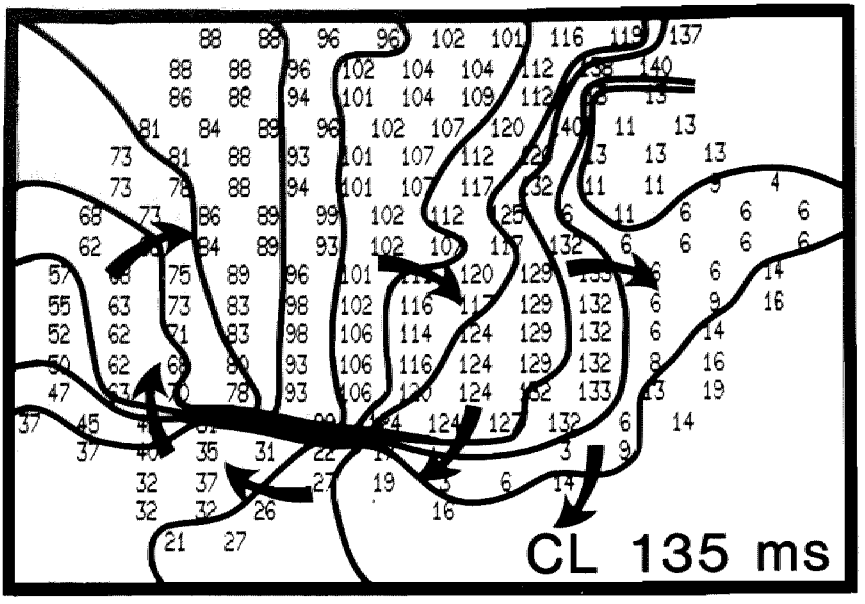
The central line of conduction block during anisotropic reentry may be caused by anatomical or functional properties of the myocardium. The results presented in chapter III demonstrated that no large anatomical barriers were present in the surviving epicardial layer and that functional anisotropic conduction block perpendicular to the fiber direction could be induced by high potassium, rapid pacing or the application of premature beats.

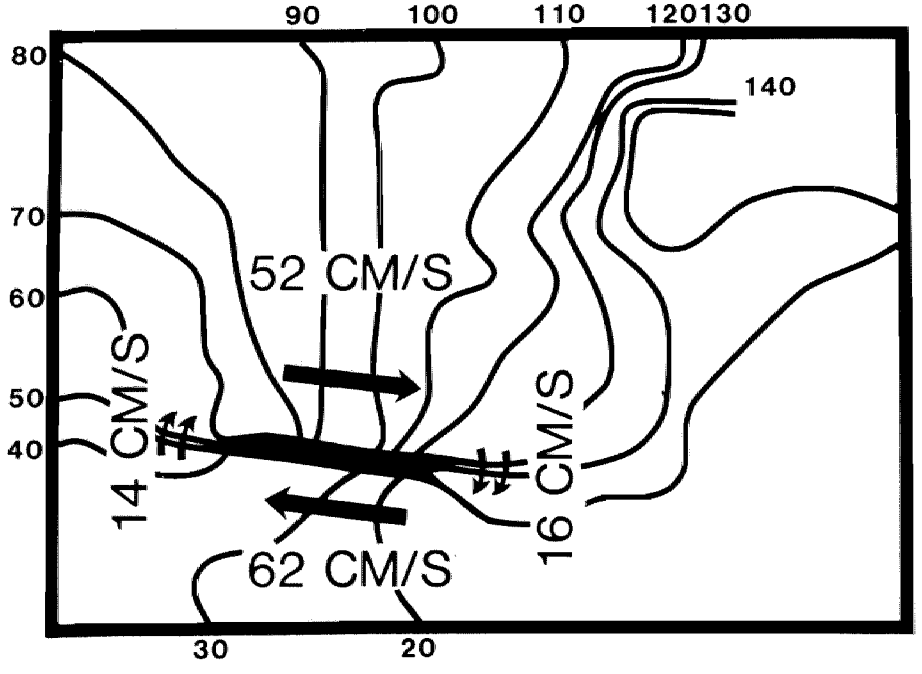
To study the properties of the line of conduction block, the electrograms recorded from the central area of the reentrant circuit were analysed.

In figure 4.8 the electrograms recorded from the central area of the circuit and the region of conduction block are plotted. The electrograms recorded from the longitudinal limbs of the circuit exhibited large biphasic deflections preceded by an R-wave. During transverse conduction at the pivoting points of the circuits the amplitude of the recorded electrograms was much smaller and no or only small R-waves preceeded the negative deflection. Electrograms recorded from the transverse line of block consisted of two distinct mainly positive deflections. These positive defections were caused by electrotonic current flow between the two opposing longitudinal activation wavefronts. The double component electrograms did not show multiple low amplitude fragmented deflections which are seen during slow transverse conduction in nonuniform anisotropic tissue (Spach et al., 1986).

Additionally extracellular electrograms at the central line of block were recorded with a very high density mapping electrode. This electrode, consisting of two rows of 48 electrodes had a spatial resolution of 0.185 mm (silver wires, 0.05 mm diameter). After localization of the epicardial circuit with the "spoon" electrode, this high resolution electrode was positioned across the line of central block.

An example is given in figure 4.9. As can be seen from the activation map, the impulse circulated around a line of transverse block with a cycle length of 135 ms. The electrograms recorded at different sites across the line of block are given in panels A,


CL 135 ms


90
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130
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20
52 CM/S
14 CM/S
16 CM/S
62 CM/S

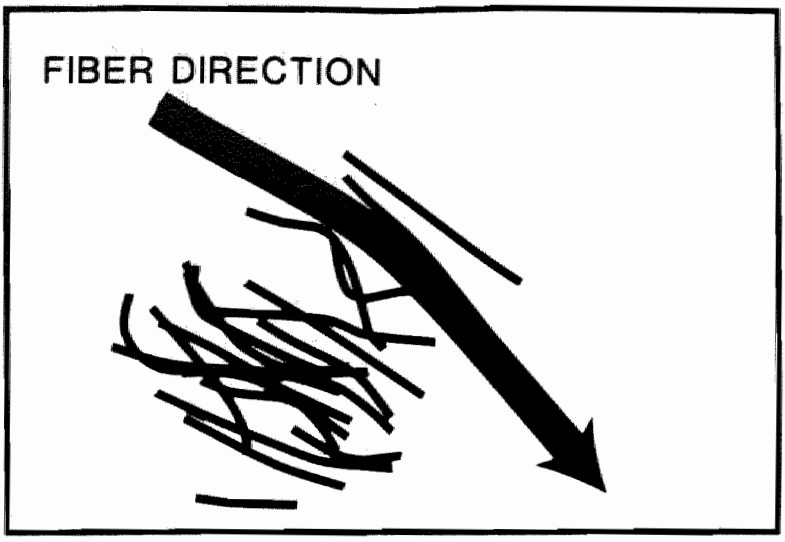
B, C. The family of electrograms displayed in panel B was recorded from the exact center of the circuit, where a difference of half the cycle length (67 ms) existed between the opposing longitudinal limbs of the circuit. A regular and single high amplitude biphasic deflection was recorded from each of the longitudinal limbs of the circuit. At the line of central conduction block the amplitude of the electrograms decreased and a second low amplitude positive deflection appeared. Electrograms recorded from the line of block showed two mainly positive deflections. Between these two deflections an isoelectrical segment was present and no multiple low amplitude deflections, indicating slow transverse conduction across the line of block were recorded. When the high resolution mapping electrode was shifted towards the pivoting points, the time difference between the double deflections shortened (panels A and C). The electrical crosstalk between the opposing longitudinal limbs of the circuit, demonstrated by these waveplots show that electrical coupling was intact and that no insulating anatomical barriers were present.

Pseudoblock.

In recent studies Wit et al. (1987) and Dillon et al.(1988) demonstrated that reentrant excitation within thin surviving epicardial layers may be the source of sustained ventricular tachycardia in the healing phase after myocardial infarction. They found that during sustained tachycardia the impulse circulated around a line of functional conduction block, which was orientated parallel to the epicardial fiber direction in most experiments. However sometimes, although the isochrones suggested the presence of a long line of block, actually slow discontinuous transverse impulse conduction (less than 5 cm/s) through the line of block occurred, as reflected by the occurrence of multiple low amplitude deflections in the extracellular electrograms. The slowing of the transverse conduction velocity was caused by the interposition of collageneous septa

Figure 4.6. Conduction velocity around the line of conduction block. Due to the anisotropy of the ventricular myocardium conduction velocity varied around the circuit depending on the direction of the impulse. During longitudinal conduction, the velocity of the impulse varied between 52 and 62 cm/s. When the impulse pivoted around the line of block, during transverse impulse conduction, the conduction velocity was as slow as 14 - 16 cm/s.

A



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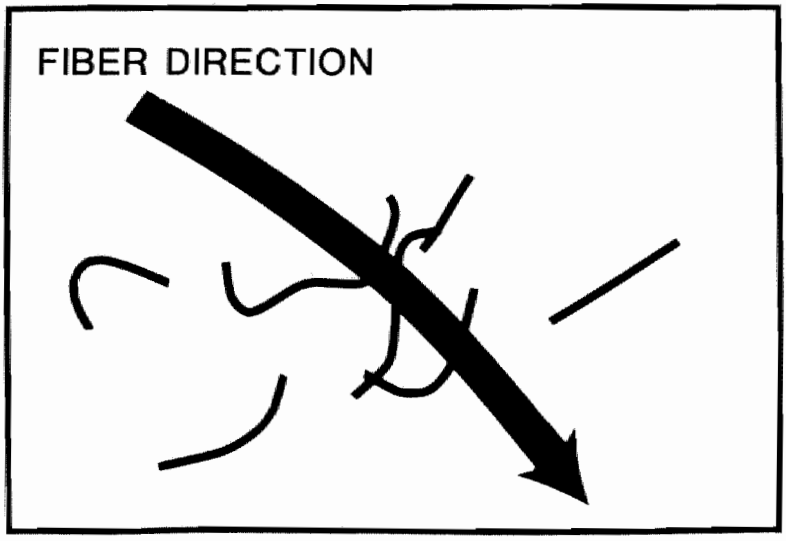
B



APEX

and destroyed cells between surviving myocardial fibers. (Wit et al., 1987, Dillon et al., 1988).

In the uniform epicardial layer a much faster transverse conduction was found (15-20 cm/s) and during most of the sustained tachycardias a reentrant circuit circulated around a true line of transverse conduction block. However in some preparations slow transverse impulse conduction across the line of apparent block could be detected. Figure 4.10 gives an example of such a "pseudoblock". The cycle length of the tachycardia was 122 ms and a single impulse circulated around a line of transverse conduction block. Fast conduction was present during longitudinal conduction while at the pivoting points the impulse propagated slowly in a transverse direction. However the tissue at one side of the line of block was activated nearly simultaneously. Consequently the 20, 30 and 40 ms isochrones are orientated parallel to the line of block. This might be explained by very slow transverse conduction across the line of block.

The spread of activation during the initiation of sustained tachycardia.

Due to the enhanced anisotropic conduction properties of the two dimensional epicardial layer, local conduction block and slow conduction could be induced by depressing the stimulating efficacy of the impulse (chapter III). Although conduction block occurred more promptly during transverse conduction, reentry was not initiated until eventually a short longitudinal line of conduction block appeared. This short line of longitudinal block forced the impulse to propagate in a transverse direction providing sufficient delay for reentry (chapter III, figure 3.13). After the initial beats the pattern of excitation changed completely however due to the limited size of the high resolution mapping electrode (13x15 mm) it was not possible to map the total spread of epicardial excitation during initiation of tachycardia.

Figure 4.7. Orientation of the lines of conduction block during sustained tachycardia. In most of the experiments (75%) the impulse circulated around a long line of conduction block orientated parallel to the epicardial fiber direction (panel A). The length of these lines of block was 20 $\pm$ 5 mm and the cycle length of the tachycardias was 129 $\pm$ 10 ms. During 25 % of the tachycardias a different orientation of the line of conduction block was recorded (panel B). The length of these lines of block was 19 $\pm$ 6 mm and the mean cycle length of these tachycardias was 123 $\pm$ 4 ms.

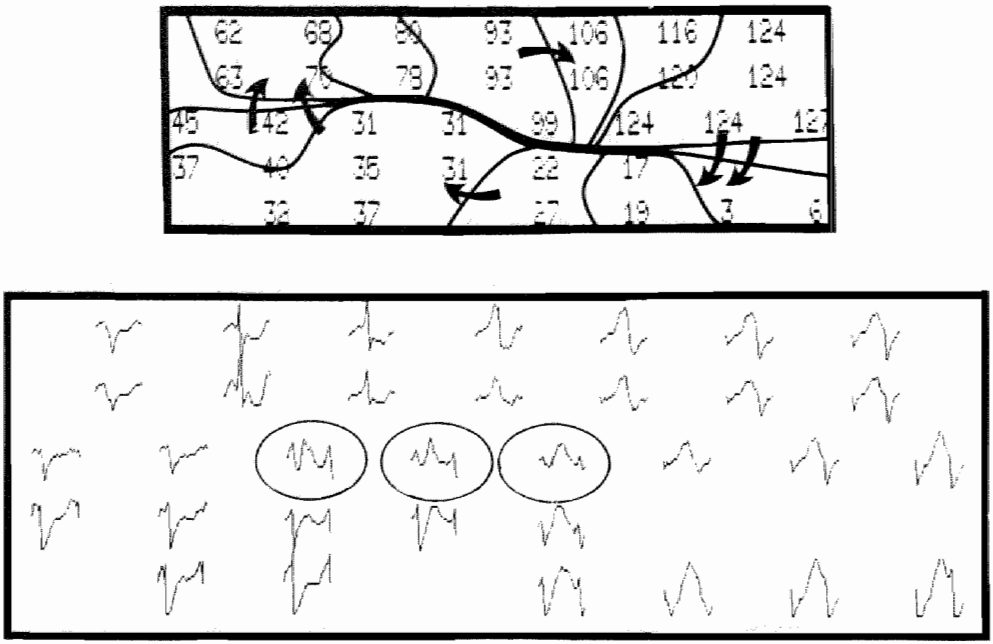


Figure 4.8. Properties of the central line of conduction block during sustained tachycardia. A large reentrant loop circulating around a long line of transverse conduction block was present. The electrograms recorded from the central circuit region of the circuit and the arc of conduction block are given. Electrograms recorded around the line of block exhibited a 1:1 response and either large biphasic deflections (during longitudinal conduction) or low amplitude deflections preceeded by a small R-wave (during transverse conduction, at the pivoting points) were recorded. The electrograms (encircled) recorded from the line of conduction block exhibited two deflections. The delay between both deflections is equal to the time difference between the adjacent limbs of the circuit at each site of the line of block.

In this paragraph we will describe the initial phase of a sustained tachycardia. By mapping the epicardial activation pattern with the "spoon" electrode, a detailed reconstruction could be made (figure 4.11).

The tachycardia was initiated by two shortly coupled premature stimuli applied during regular pacing with a cycle length of 350 ms (top tracing). In panels S1, S2, and S3 the activation maps recorded during regular pacing (S1) and the two premature beats (S1-S2 = 155 ms, S2-S3 = 135 ms) are given. The heart was stimulated from a pair of electrodes located in the upper left part of the mapping electrode. During regular pacing an ellipsoid activation pattern was recorded. The impulse conducted rapidly parallel to the epicardial fibers and more slowly perpendicular to the fiber direction. No signs of impaired conduction or conduction block were found. During the first premature beat (S2) longitudinal and transverse conduction were slowed down slightly.

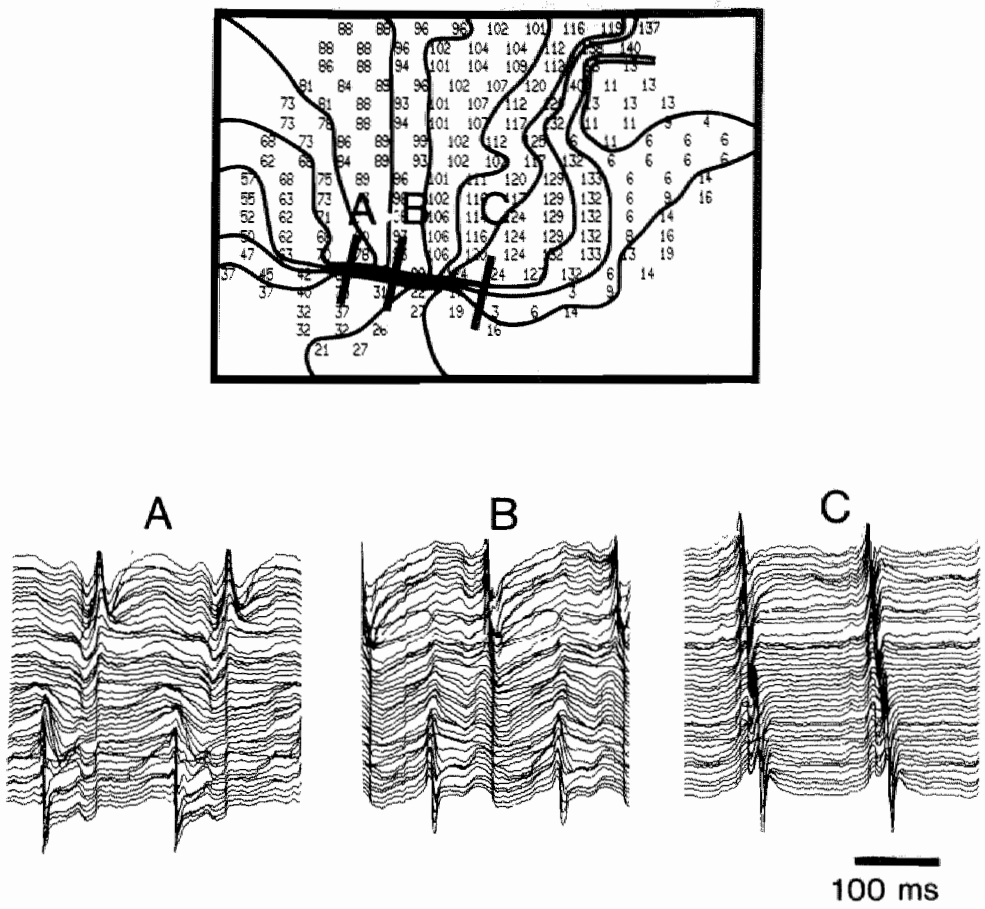


Figure 4.9. Wave plots recorded during sustained tachycardia. The activation map showed that the impulse circulated around a line of transverse block with a cycle length of 135 ms. The electrograms recorded at different sites across the line of block are given in panels A, B, C. See for explanation text

A short line of transverse conduction block emerged near the site of stimulation (fusion of isochrones). During the second premature beat (S3) conduction was further depressed, both in a longitudinal and transverse direction and a few lines of transverse and longitudinal conduction block appeared. About 140 ms after the second premature stimulus, a spontaneous beat originated near the orginal site of stimulation (VT1). Because of the relatively low spatial resolution of the "spoon" electrode it was not possible to decide whether this first spontaneous beat was initiated by spontaneous

PSEUDO BLOCK

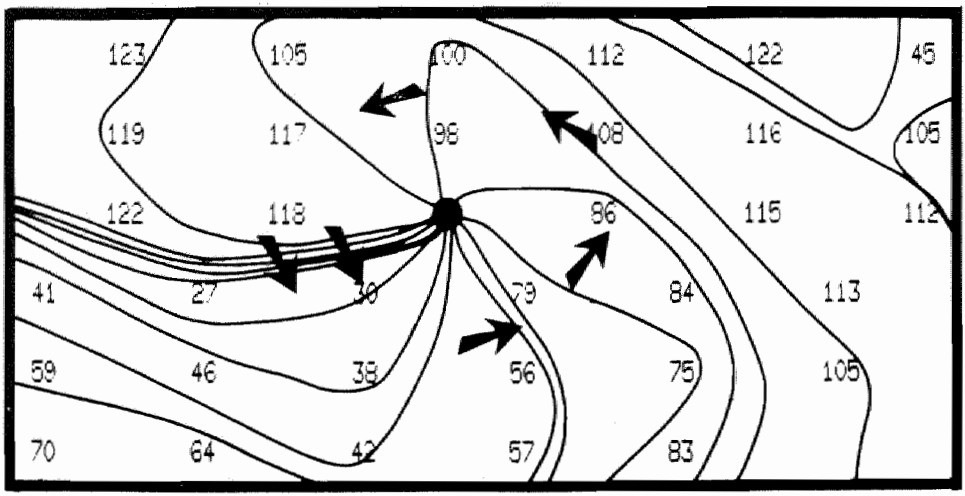
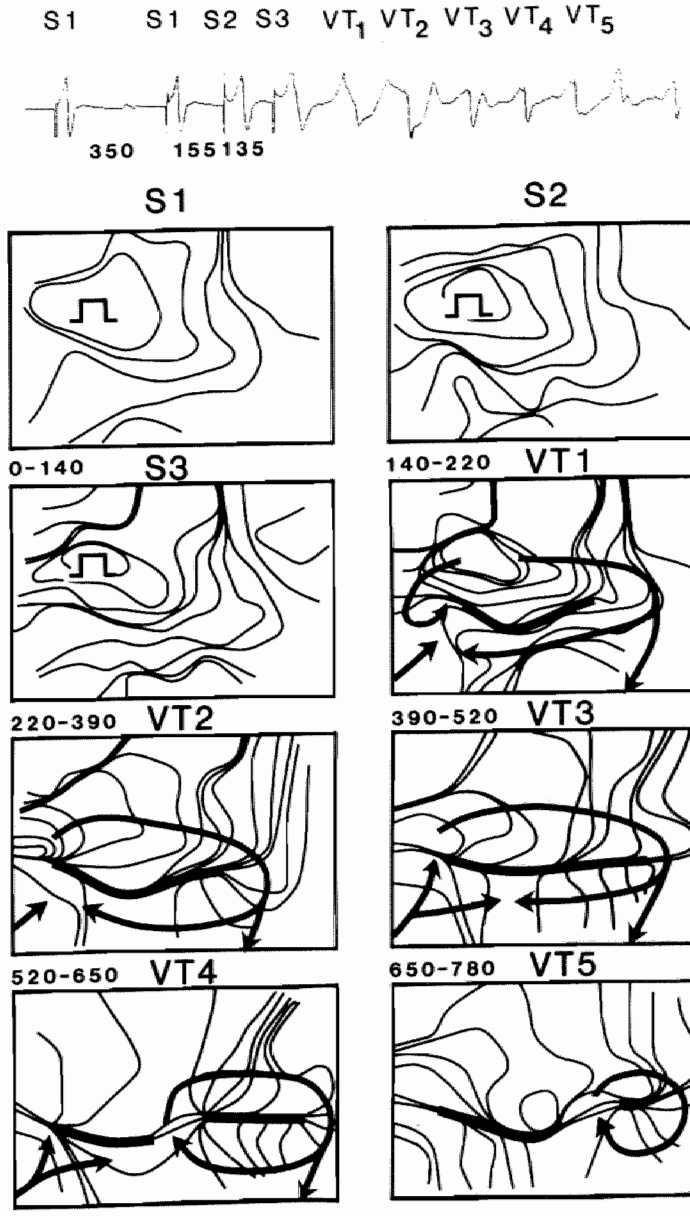


Figure 4.10. Pseudo block. The activation map showed the central circuit area during sustained tachycardia. The cycle length was 122 ms and the impulse conducted around an apparent line of transverse conduction block. Near the line of block large parts of the myocardium were activated nearly without any conduction delay. The 20, 30 and 40 ms isochrones are orientated parallel to the line of block and extend along the line of block.

impulse formation or by reentrant excitation within a small region near the site of stimulation. However the activation map of the first beat of the tachycardia showed that conduction became more depressed and that some lines of conduction block emerged. The impulse pivoted around both ends of a relatively long line of conduction block in the center of the map. After about 80 ms the second spontaneous beat originated when the impulse propagated retrogradely through the line of block near the left pivoting

Figure 4.11. The initiation of sustained tachycardia by two closely coupled premature beats. The isochrone maps during regular pacing (S1), premature stimuli (S2-S3) and the first five beats of the ensuing ventricular tachycardia are shown (VT1-VT5). See for further explanation text.

INITIATION OF TACHYCARDIA



point (VT2). The impulse propagated in a clockwise direction around the long line of conduction block. Because the length of the line of block was increased the length of the excitation pathway increased also and as a result of this the next spontaneous beat (VT3) originated only after about 170 ms when the impulse conducted around the left end of the line of block. During this beat the impulse circulated again around the long line of conduction block, but was partially blocked by an impulse circulating around the apex. At the site of collision of these two wavefronts the impulse now succeeded to propagate up through the line of conduction block thus short circuiting the left part of the circuit. Consequently the circuit during the next beat (VT4) had smaller dimensions and shifted to the right. During the next beat the length of the line of conduction block was further shortened (VT5). This pattern of excitation found during the fifth beat of the tachycardia remained stable, during the whole period of the tachycardia.

CHAPTER V

GENERAL DISCUSSION

Due to the anisotropic morphology of the ventricles, conduction is about three times faster during impulse conduction parallel to the fiber axis, compared to transverse conduction. Consequently the wavelength of refractoriness, given by the product of conduction velocity and refractory period is three times longer during longitudinal conduction than during transverse conduction (chapter II). Due to the three dimensional geometry of the ventricles the arrhythmogenic effects of slow transverse conduction and the short transverse wavelength are masked by a fast and homogeneous impulse spread in nearly all different directions (chapter III). Because of this, in normal intact ventricles, ventricular tachycardias do not occur. After removal of the intramural and endocardial layers of the rabbit ventricle the effective distance of slow transverse impulse conduction increased and local conduction block could be easily initiated (chapter III). It emerged that anisotropic conduction and conduction block played a key role in the initiation of sustained reentrant tachycardia in a thin epicardial layer. During sustained tachycardia, the impulse circulated around a line of functional conduction block; no gross anatomical barriers were involved in the reentrant circuit (chapter IV). However in contrast to the leading circle type of functional reentry (Allessie et al., 1977), in uniform anisotropic myocardium a clear excitable gap was present. Due to the presence of an excitable gap, anisotropic reentry was more stable and longlasting.

In this chapter the characteristics of "anisotropic" reentry and the mechanisms contributing to the creation of an excitable gap will be discussed.

5.1. INITIATION OF ANISOTROPIC REENTRY.

Among other things the inducibility of reentrant arrhythmias is determined by the wavelength of refractoriness (Smeets et al., 1986, Rensma et al., 1988, Lammers et al., 1988). Recently Rensma et al. (1988) demonstrated that the wavelength of refractoriness is a sensitive and specific index of the susceptibility of the heart to atrial arrhyth-

mias. A short wavelength was found to be a risk factor for the initiation of reentry. A long wavelength on the other hand, could prevent the induction of reentry. In agreement with this we demonstrated that in the intact ventricle, due to the fast three dimensional impulse spread, the wavelength was long and no ventricular fibrillation or tachycardia could be initiated by the application of shortly coupled premature stimuli. Only after prolonged periods of maximal pacing, when conduction has become seriously depressed and consequently the wavelength was shortened, ventricular fibrillation was initiated. In the two dimensional epicardial layer slow transverse impulse conduction was no longer bypassed via faster conducting deeper layers and as a result the area of effective anisotropy increased. When the stimulating efficacy of the impulse was lowered and conduction block developed, sustained ventricular tachycardia could be initiated after the application of two or more shortly coupled premature stimuli (chapter III and IV). Ventricular tachycardia was never observed after induction of only one premature beat. This is explained by the fact that the wavelength of a single premature beat was only slightly shorter than during regular pacing and no conduction block occurred.

The minimal length of the line of conduction block required to initiate reentry has to be at least half the length of the excitation wave. When the line of block is orientated perpendicular to the fiber direction (e.g. longitudinal conduction block) the impulse activates the myocardium distal to the line of block in a slow transverse manner at a conduction velocity of about 20 cm/s. During fast pacing (for instance 100 ms cycle length) the transverse wavelength is 2 cm and a block of 1-2 cm will be required to initiate reentry. In nonuniform anisotropic myocardium, when transverse conduction may be slowed to less than 5 cm/s, the minimal length of the line of block might be even less than 0.5 cm (Spach et al. 1981, 1986). However when the line of block is positioned parallel to the epicardial fiber direction (transverse conduction block) the myocardium distal to the line of block will be activated by fast longitudinal conduction (60 cm/s), and the minimal length of the line of block for reentry will be about 6 cm. The initiation of reentry thus will be facilitated by both the occurrence of longitudinal conduction block and slow transverse impulse conduction. In agreement with this we demonstrated (chapter III) that although conduction block occurred more promptly during transverse conduction, reentry was rarely initiated unless also an area of longitudinal block occurred.

5.2. SUSTAINED "ANISOTROPIC" REENTRY

The cycle length of sustained ventricular tachycardia ranged from 105 ms to 160 ms (mean 130 11 ms) with an excitable gap of 10 to 40 ms. Reentrant excitation was found to be the underlying mechanism of sustained tachycardia. Due to the anisotropy of the ventricular myocardium (Clerc, 1977, Spach et al., 1981, 1982) the conduction velocity of the circulating impulse around the circuit varied from about 60 cm/s during longitudinal conduction to less than 20 cm/s during slow transverse conduction. The presence of an excitable gap explained the stability and long duration of the tachycardias.

At the line of block no insulating anatomical barriers could be found and the size of the circuit will thus be determined by the electro-physiological properties of the myocardium (chapter IV). The length of the circular pathway is determined by the product of the average conduction velocity and the cycle length of the arrhythmia. When the line of conduction block is orientated parallel to the epicardial fiber direction, the mean conduction velocity around the circuit is about 40 cm/s. During a tachycardia with a cycle length of 130 ms, the length of the circular pathway will then be 5.2 cm. The surface area required for the perpetuation of a reentrant tachycardia will be 2.3 cm^2. In nonuniform anisotropic myocardium transverse conduction may slow down to less than 5 cm/s (Spach et al., 1981, 1982a, 1986). This can decrease the mean conduction velocity to less than 19 cm/s and the circumference of the circuit to less than 2.5 cm, under these circumstances a surface area of only 0.5 cm^2 would suffice for a circus movement of the impulse.

When the line of block is orientated perpendicular to the fiber direction in uniform anisotropic myocardium, the mean conduction velocity is estimated to be 25 cm/s. In this situation the minimal circumference of the circuit will be 3.3 cm, resulting in a surface area of only 0.9 cm^2. In addition, when transverse impulse conduction is further slowed down in nonuniform anisotropic myocardium, to less than 5 cm/s, the circumference of the circuit may shorten to less than 1 cm, resulting in a minimal surface area of only 0.08 cm^2.

The minimal surface area necessary for the maintaince of anisotropic reentry thus varies from about 2.3 cm^2 when the impulse circulates around a transverse line of conduction block to less than 0.08 cm^2 when the impulse circulates around a longitudinal conduction block in nonuniform anisotropic myocardium. This is in accordance with

the suggestion of Spach et al. (1981) that in nonuniform anisotropic myocardium micro reentrant circuits may be confined within only a few square millimeters. Experimental results of Wit et al. (1987) and Dillon et al. (1988) demonstrated that reentrant circuits within the borderzone of the infarct may cause sustained ventricular tachycardias. Due to slow discontinuous transverse impulse conduction some of these circuits were extremely small.

The rate of circus movement excitation depends on the length of the circular pathway and the average conduction velocity around the circuit. During functional reentry the circulating impulse follows the shortest possible pathway and the circuit length is equal to the length of the excitation wave (Allessie et al., 1977). Consequently the revolution time will be determined by the functional refractory period. During anisotropic reentry however, due to the presence of an excitable gap, the circuit length is not equal to the length of the excitation wave. As a consequence, the rate of anisotropic reentry will be determined by the functional refractory period add to the duration of the excitable gap.

In conclusion the characteristics of "anisotropic" reentry are:

1. A central line of functional conduction block mostly parallel to the fiber orientation.
2. Varying conduction velocities of the circulating impulse caused by changes in direction relative to the fiber direction.
4. The presence of an excitable gap.
5. A cycle length proportional to (but longer than) the refractory period of the myocardium.
6. A high degree of stability.

Anatomical, functional and anisotropic reentry.

A number of important differences exists between anisotropic reentry and either anatomical or leading circle reentrant arrhythmias. 1. In contrast with anatomical reentry no insulating barrier is present and the line of block consists of normal cells during leading circle and anisotropic reentry. 2. Whereas the size of an anatomical circuit is determined by the size of the anatomical barrier, during leading circle reentry and during anisotropic reentry the length of the circular pathway is determined by the electrophysiologic properties of the myocardium. 3. During leading circle reentry and dur-

ing anisotropic reentry the circuit length changes with variations in conduction velocity and refractory period, while during anatomical reentry the length of the circular pathway is fixed. 4. During anatomical and anisotropic reentry an excitable gap is present, while during leading circle reentry no excitable gap is present because there exists a tight fit between the crest and the tail of the impulse. Consequently anatomical and anisotropic reentry are stable and longlasting whereas leading circle reentry tends to terminate spontaneously. 5. The rate of a leading circle reentry is equal to the refractory period whereas due to the presence of an excitable gap the rate of anisotropic reentry is proportional to the refractory period of the myocardium and the duration of the excitable gap. During anatomical reentry the rate of the tachycardia is inversely related to the conduction velocity.

Characteristics of anatomical, functional and anisotropic reentry.

Anatomical	**Leading circle**	**Anisotropic**
1.Impulse circulating around an anatomical barrier.	1.Functional conduction block	1.Functional conduction block
2.Large dimensions	2.Smallest possible dimensions	2.Intermediate dimensions
3.Fixed pathway	3.Size of the circuit may change with alterations of electrophysiologic properties	3.Size of the circuit may change with alterations of electrophysiologic properties
4.Excitable gap	4.No excitable gap	4.Excitable gap
5.Stable and longlasting	5.Tends to terminate spontaneously	5.Stable and longlasting
6.The revolution time inversely related to conduction velocity	6.Revolution time proportional to refractory period	6.Revolution time proportional to refractory period and excitable gap

5.3. THE EXCITABLE GAP

The presence of an excitable gap during anisotropic reentry is the most important difference compared to leading circle reentrant excitation (Allessie et al., 1977). The existence of an excitable gap of 10-40 ms in a functionally determined circuit, might be explained by three possible mechanisms (figure 5.1).

Microanatomical barriers at the pivoting points.

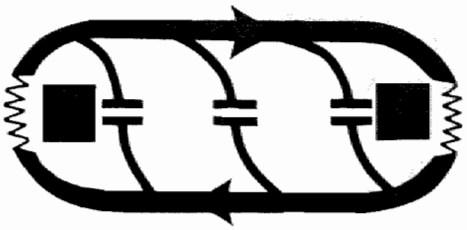
Although no gross anatomical barriers are involved, the presence of microanatomical obstacles at the pivoting points of the circuit may enlarge the central, functionally determined line of block of the circuit. It will also stabilize the position of the reentrant loop at a fixed location in the myocardium. Small conduction barriers may exist when, due to the interposition of collageneous septa, adjacant myocardial fibers become separated (Sommer and Dolber, 1979, Sommer, 1983). Such microanatomical barriers at the pivoting points of the circuit lengthen the functional conduction block, thus creating an excitable gap during tachycardia. The dimensions of such a reentrant circuit are determined by the distance between these barriers.

Block at the pivoting points because of increased electrotonic current load.

Spatial differences of the stimulating efficacy of the impulse, due to the effects of tissue anisotropy, may contribute to the creation of an excitable gap. The stimulating efficacy of a propagating action potential is influenced by sudden changes in the axial current load (Spach et al., 1981, 1982a, 1986, 1987). Such a sudden increase of the current load occurs during branching of myocardial fibers or when an abrupt change in the direction of impulse propagation occurs. A depression of the stimulating efficacy

Figure 5.1. Three possible mechanisms contributing to the creation of an excitable gap are schematically summarized.
1: Microanatomical barriers at the pivoting points may lengthen the line of functional conduction block.
2: A sudden depression of the stimulating efficacy of the impulse at the pivoting points due to a sudden increase of the axial current.
3: Action potential prolongation at the pivoting points prevents the impulse from short circuiting the circuit.

MICROANATOMICAL



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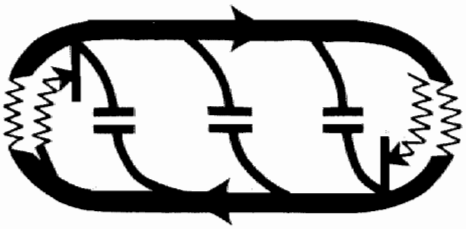
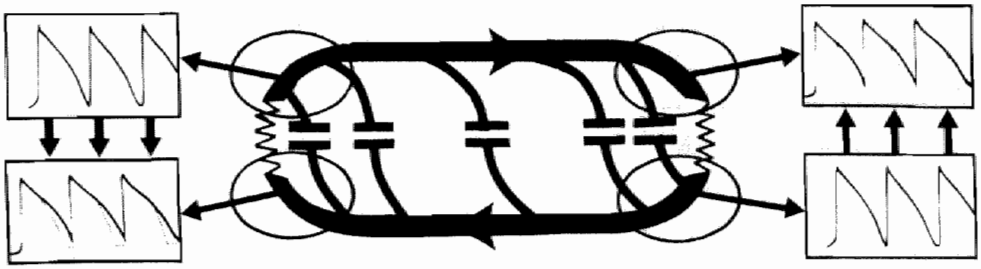


ACTION POTENTIAL PROLONGATION



may lead to decremental conduction or conduction block despite the fact that the cells are excitable.

At the pivoting points of anisotropic reentry, the slowly conducting transverse wavefront encounters a sudden increase of axial current load when the returning longitudinal limb has to be activated. It is possible that at the transition from transverse to longitudinal conduction the sudden increase of electrotonic load leads to temporary conduction block. The returning longitudinal limb of the circuit will not be activated until a larger part of the wavefront has rotated around the pivoting point. This temporal halt of the impulse at the pivoting points may create an excitable gap because it results in a functional lengthening of the central line of conduction block and consequently in a prolongation of the cycle length of the tachycardia.

Electrotonic prolongation of action potential duration at the pivoting points.

Spatial differences of the action potential duration might also contribute to the creation of an excitable gap. It is known that during regular pacing the action potential duration near the stimulation site is prolonged by electrotonic interaction with more remote cells which are activated later in time (Hoffman and Cranefield, 1960, Osaka et al., 1987).

Recently Osaka et al. (1987) demonstrated that the amount of prolongation of the action potential duration depends on the conduction time between the cells and the axial resistance. During centrifugal impulse spread in anisotropic myocardium the prolongation of the action potential duration was most marked. The prolongation of the action potential duration lengthened the local refractory period.

During anisotropic reentry considerable differences in activation time are present around the pivoting points. Because electrical coupling is intact, these differences in activation time over only a few millimeters may result in action potential prolongation of the cells proximal to the pivoting point and consequently in a lengthening of the local refractory period. This selective prolongation of refractoriness at the pivoting points will delay the moment the impulse can turn around, thus lengthening the central line of conduction block. Because of local prolongation of refractoriness, at the pivoting points there is a tight fit between the crest of the depolarization wave and its tail of refractoriness. However in all other parts of the heart an excitable gap will exist during anisotropic tachycardia.

5.4. CELLULAR CHARACTERISTICS OF ANISOTROPIC REENTRY.

Preliminary results of a study of the cellular characteristics of anisotropic reentry show clear electrotonic interaction across the central line of conduction block and at the pivoting points. Standard microelectrode techniques were used and in figures 5.2-5.4 action potentials recorded from different parts of the circuit are given. During sustained ventricular tachycardia a regular 1 to 1 activation response and normal action potentials were recorded from different sites outside the line of conduction block. An alternans in action potential duration and in action potential amplitude was frequently observed. In the central area of the circuit variable degrees of conduction block were recorded. In figure 5.3 an example of 2 to 1 conduction block is given. As can be seen the action potential duration in the center of block was clearly prolonged by a electrotonic interaction between the two longitudinal limbs of the circuit. Not only 2 to 1 block but also local 3 to 2 conduction block was sometimes observed. The possible mechanism of 3 to 2 conduction block is explained in figure 5.4. Due to a slight prolongation of the action potential duration, the cells were activated every other cycle by one of the opposing longitudinal wavefronts. The strong electrotonic interaction found between the adjacent limbs of the circuit may also prolong the action potential duration at the pivoting points and contribute to the creation of an excitable gap during functional reentry in anisotropic myocardium.

5.5. COMPUTER SIMULATION OF ANISOTROPIC REENTRY.

Computer simulation studies may contribute to the elucidation of the role of tissue anisotropy in determining the characteristics of functional reentrant excitation and the creation of an excitable gap. Recently Lammers et al. (1987) demonstrated, that when in a simple computer simulation model, consisting of 900 cells, arranged in a regular matrix of 30 x 30 elements, the properties of the sheet were changed from isotropic to anisotropic, during circus movement excitation, an excitable gap appeared. However in this model, the actual transmembrane currents could not be calculated. Therefore a more suitable model was used to simulate the effects of tissue anisotropy on conduction and action potential configurations. This model, based on actual transmembrane currents, was developed by Dr F.J.L. Van Capelle, department of experimental

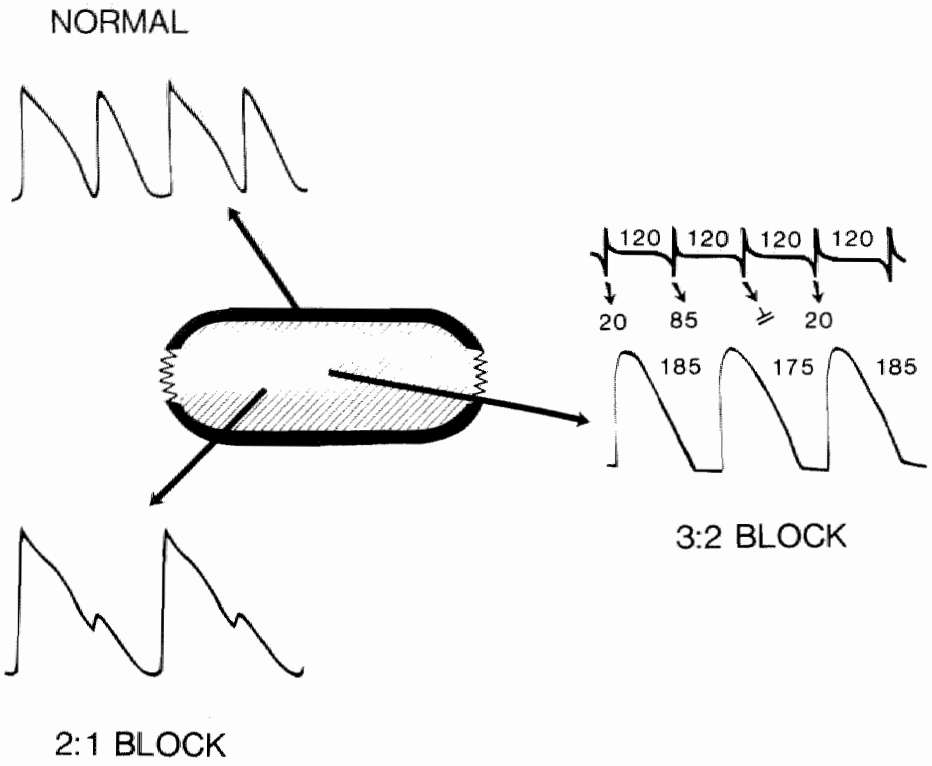


Figure 5.2. Cellular characteristics of anisotropic reentry. Action potentials recorded from different parts of the circuit area are shown. Normal action potentials were recorded from different sides around the circuit. Action potentials recorded from the central circuit area reflected variable degrees of functional conduction block.

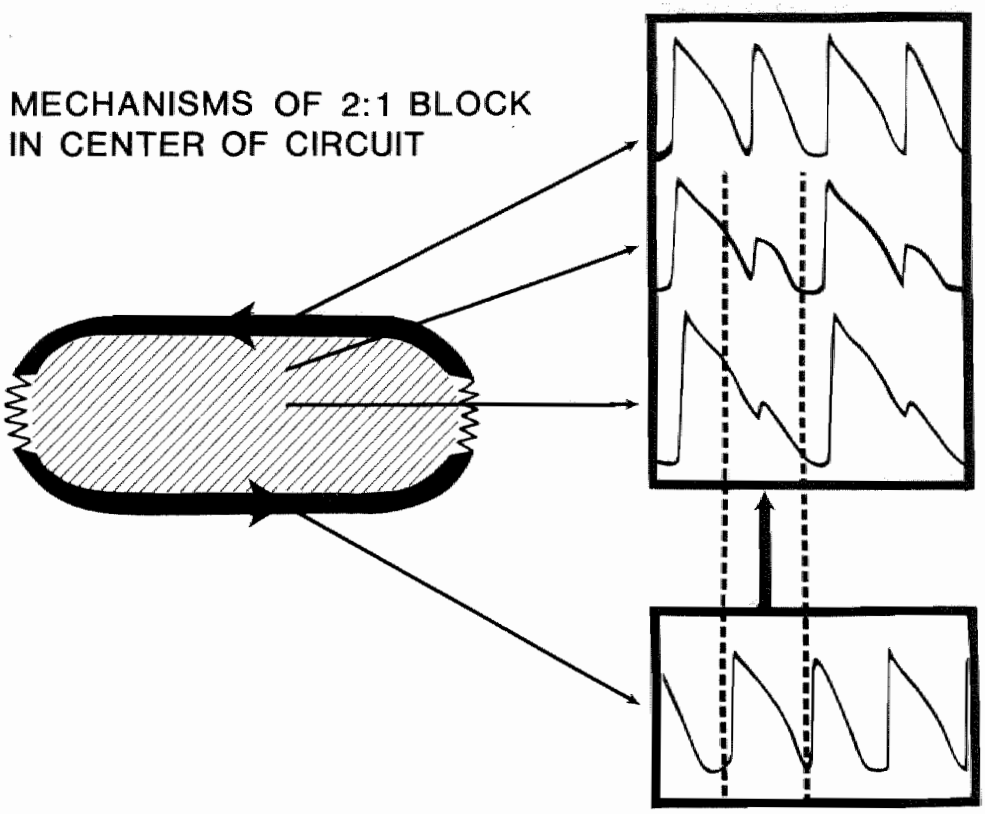
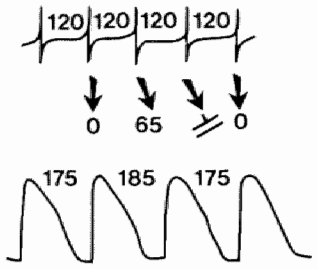
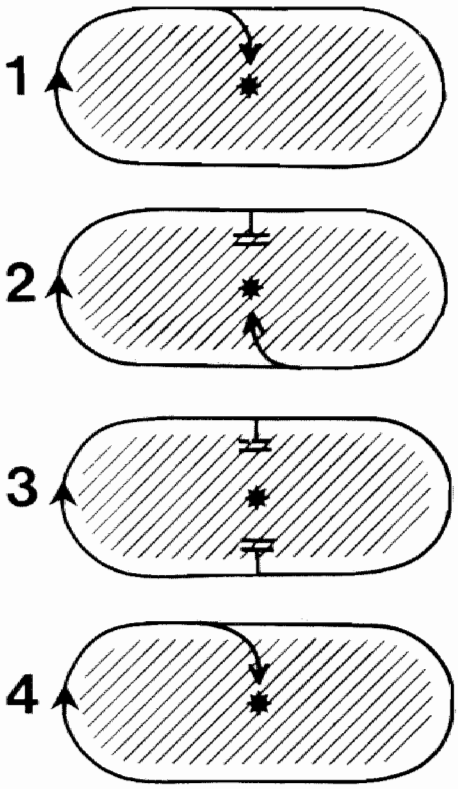


Figure 5.3. In the center of the circuit due to electrotonic interaction between the longitudinal limbs of the circuit the plateau phase of the action potentials was prolonged. This electrotonic prolongation of the action potentials caused conduction block in the center of the circuit. As a result in the center 2 to 1 conduction block was found, and the same mechanism may cause action potential prolongation at the pivoting points of the circuit, thereby creating an excitable gap (see text for further explanation).

3:2 BLOCK IN CENTER OF CIRCUIT





cardiology, University of Amsterdam. The simulation study was performed in co-operation with Dr Van Capelle.

Before presenting the results of this study, first a short summary of the essential characteristics of the computer simulation as described by Dr van Capelle (Van Capelle and Durrer, 1980) model will be given.

"The model consists of up to 650 excitable elements, arranged in a geometry which is to be specified at the start and which may be changed during execution of the simulation. As an example, a square lattice geometry is illustrated in figure 5.5. The elements are coupled through passive resistances, which may be set individually to arbitrary values (although no more than five different values are accepted by the program). The elements need not be all identical. Elements may be deleted or changed at any time during simulation, and coupling resistances may be deleted, changed, or added between any two elements. The network of resistors overlying the elements in figure 5.5 may be thought of as representing the intracellular space. The lower side of the elements is connected to the extracellular space, which is assumed to be zero potential at all times.

From the instantaneous transmembrane potentials of the elements and from the values of coupling resistances, the currents through the intracellular maze are known. Thus the currents emerging from the elements at the nodes of the network can be calculated. Subtraction of the ionic currents flowing through the same elements at that point in time then yields the capacitive current flowing through that particular element. In this way the rate of rise of the action potentials of the elements can be calculated, and integration of this quantity yields the time course of the transmembrane voltage of all elements.

Before starting a simulation, it is necessary to define the types of elements to be used. In our simulation, this was done by specification of two membrane voltage-current relations, a steady state inactivation function, and a few parameters. An adequate choice of these quantities could result in elements possessing the desired electrophysiological characteristics. A separate interactive program which displays the relevant functions and parameters, together with the resultant action potentials and voltage

Figure 5.4. Sometimes 3 to 2 conduction block may be present in the center of the circuit. Due to a prolongation of the action potentials, the cells in the center could only be activated after one and an half cycle length. The mechanism of 3 to 2 conduction block is shown (see text for further explanation).

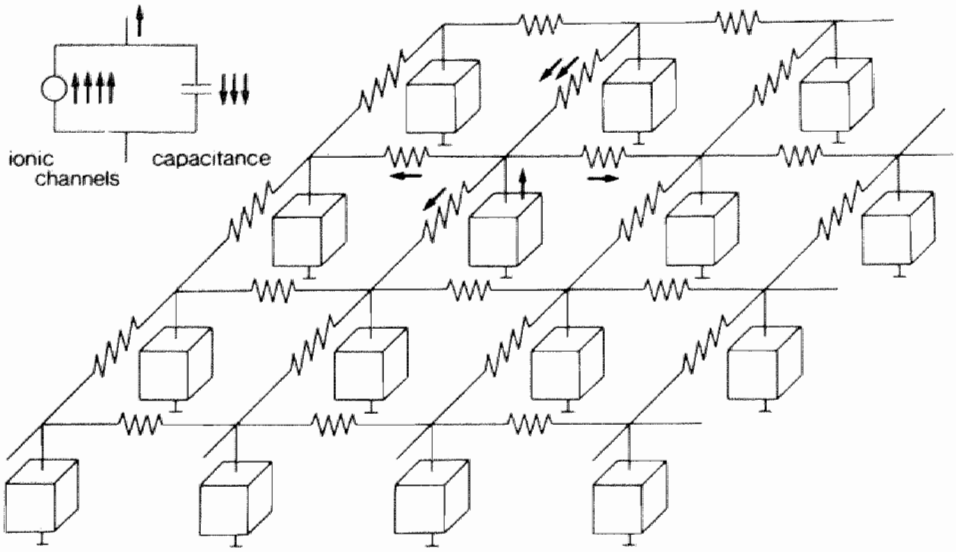


Figure 5.5. Example of arrangement of excitable elements. The bottom side of the individual elements (extracellular space) is always at zero potential. The top side is connected by a resistive network, which represents the intracellular space. The current emerging from an element consists of an ionic and a capacitive component (inset). This current matches the sum of the currents flowing to the element through the intracellular space.

clamp currents, facilitates adjustments of the properties of the elements. In this way, a library of excitable elements was created from which a selection could be made when a simulation was performed.

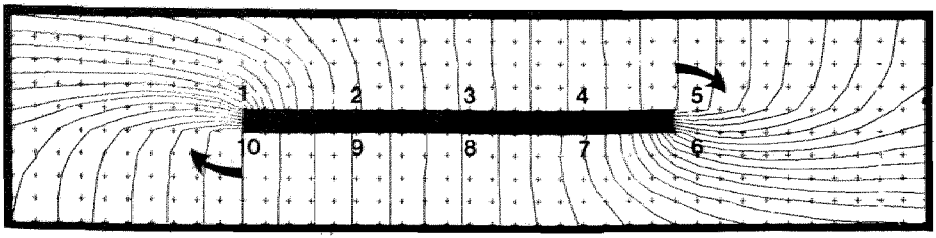
Next the arrangements of the elements must be specified. After specification of the dimensions of the sheet, the graphic terminal will initially display in a square lattice containing the elements. Now, pointing at the elements with a joystick, individual cells may be deleted, leaving a "hole" in the sheet. In the same way, existing cell connections may be deleted, and new ones installed, resulting in a network of arbitrary complexity. Using the same technique, the cell type of individual elements and the resistance of individual cell connections may be changed. Since it is confusing to display more than 10 traces simultaneously on the terminal, a selection of elements to be

monitored on the terminal is made. This is equivalent to the procedure of positioning of the recording electrodes in an actual experiment. In the same way, the stimulating sites are selected with the joystick.

After specification of the stimulus sequence, the simulation can be started. While the simulation progresses, the action potentials of the selected elements are displayed on the graphic terminal and stored on a disk. Whenever an element is activated, the activation time also is written on a separate disk file, yielding a complete survey of the activation sequence." A more detailed description of the current voltage relationships used in this model can be found in the paper of Van Capelle and Durrer (1980).

Results.

To simulate the effects of tissue anisotropy in a sheet of 400 (40x10) excitable elements, the resistance between the elements was changed in such a manner that a longitudinal and transverse axis were created with a ratio of 4 to 1 between the transverse and longitudinal resistance. The active properties of all 400 excitable elements were identical. In the center of this sheet of excitable elements a long line of "anatomical" conduction block parallel to the longitudinal axis ("transverse" conduction block) was created by deleting the transverse connections between 40 opposite elements. Reentry was initiated by the application of a single stimulus near the upper left edge of the line of conduction block. The line of block was temporarily extended to the left border of the sheet resulting in unidirectional conduction block. As a result of this, the impulse propagated in a clockwise fashion around the long line of conduction block in the center of the sheet. Just before the "returning" wavefront reached the left pivoting point of the line of block, the extended line of conduction block was removed by connecting the cells again, and as a result of this, the elements distal to the temporarily line of conduction block were reactivated. The impulse continued to circulate around the "anatomical" line of block and stable sustained tachycardia was initiated. The impulse propagated rapidly along the longitudinal axis of the circuit, whereas at the pivoting points, conduction velocity slowed down because of transverse propagation. The simulated action potentials recorded from different sites in the simulated anatomical circuit are given in figure 5.6. Normal action potential configurations were recorded during longitudinal impulse conduction. Because the length of the circular pathway exceeded the wavelength an excitable gap was present, and the excitability of the cells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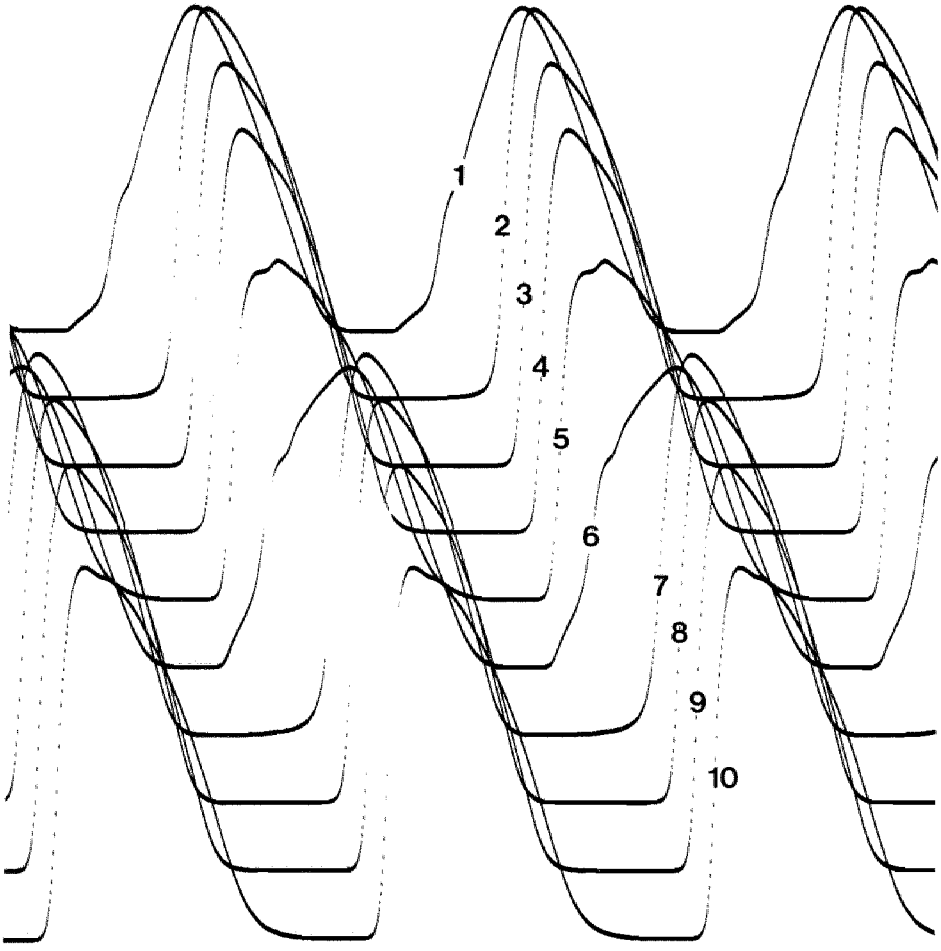
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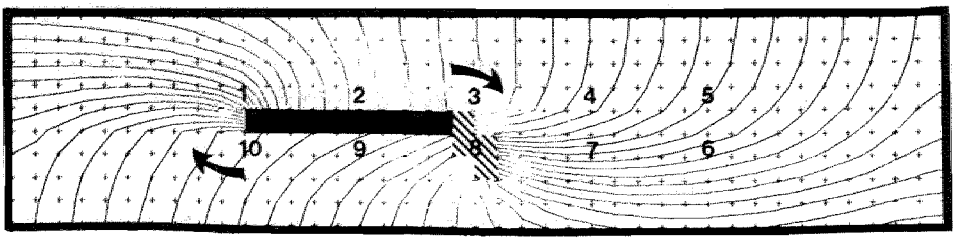
was restored before the next impulse arrived. At both pivoting points a notch was found in the upstroke of the action potentials and the upstroke velocity of the action potentials was depressed. These effects can be explained by the sudden increase of the axial current load occurring when the impulse turned circulated the pivoting points. To simulate functional reentrant excitation, the anatomical line of block was shortened at one side (right), by connecting 10 opposite cells again when the impulse rotated around the other pivoting point. In the resulting circuit one pivoting point was anatomically defined, while the right turning point was completely determined by the functional properties of the excitable elements. Figure 5.7 gives the isochronic map recorded during the resulting stable tachycardia. Compared to the fully anatomically defined circuit (fig 5.6) the length of the line of block was decreased significantly and the cycle length of the tachycardia shortened. As can be seen the action potentials recorded from the anatomical part of the circuit (elements 1,2) were not changed compared to those recorded during anatomical reentry. However a more complex action potential configuration was recorded from the functional pivoting point (element 8). Because the electrical coupling between the cells was restored, the impulse was able to conduct through the line of block and as a result, the length of the line of block was markedly shortened. However no full blown action potentials were recorded from that site and conduction block still occurred. The occurrence of conduction block at the functional pivoting point can be explained by the sudden increase of the current load due to the transition from transverse to longitudinal conduction. Such functional lengthening of an anatomical line of conduction block contributes to the creation of an excitable gap in the resulting circus movement. Due to the long duration of the action potential distal to the pivoting point (element 8) the action potential duration proximal to the pivoting point was prolonged (element 3). This prolongation may also contribute to the creation of the excitable gap in a functional reentrant circuit in anisotropic myocardium.

Figure 5.6. Computer simulation of anatomical reentry in a two-dimensional sheet of anisotropic "tissue". The impulse propagated rapidly along the longitudinal axis of the circuit, whereas at the pivoting points, conduction velocity slowed down. The numbers given in the activation map correspond to the "recorded" action potentials. At both pivoting points (elements 1 and 6) a notch was found in the upstroke of the action potentials and the upstroke velocity of the action potentials was slowed down. This is caused by a sudden increase of the axial current load when at the pivoting points the impulse suddenly changes from transverse to longitudinal conduction.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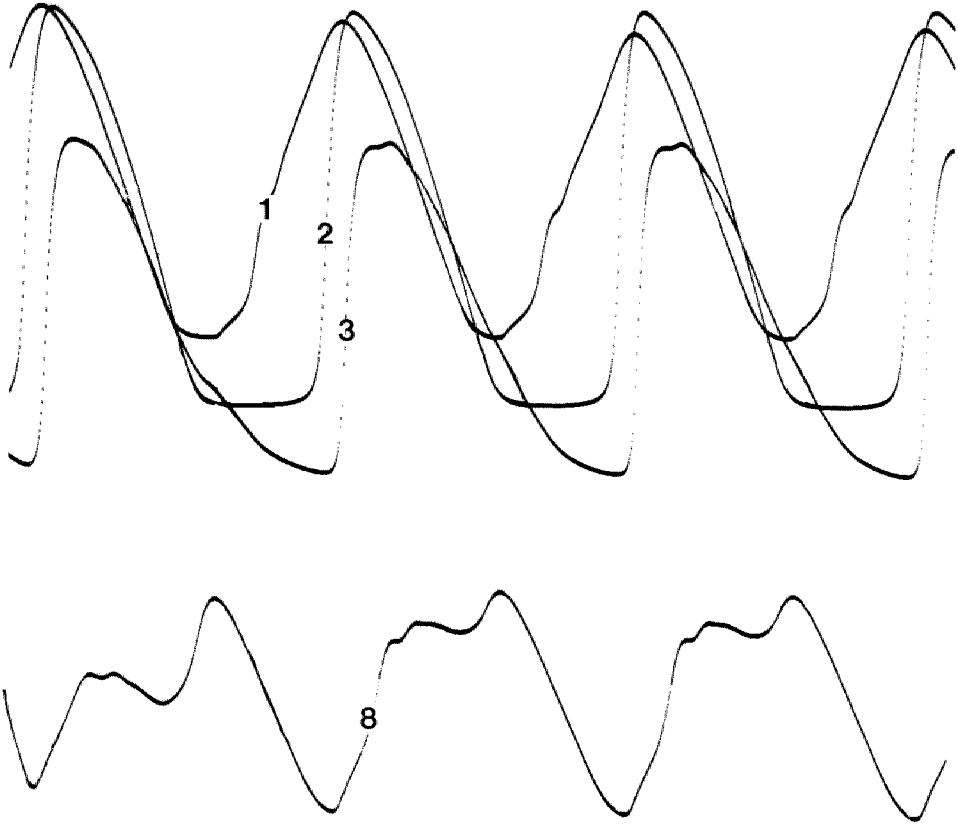
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The results of both experimental microelectrode studies and computer simulation studies emphasize the importance of passive electrotonic interaction at the pivoting points of a reentrant circuit. The results of the computer simulation studies also demonstrated, that electrotonic action potential prolongation at the pivoting points, during circus movement arrhythmias may create an excitable gap.

5.6. ANISOTROPIC REENTRY AS A MECHANISM OF SUSTAINED VENTRICULAR TACHYCARDIA IN THE POST MYOCARDIAL INFARCTION PERIOD.

Sustained ventricular tachycardia is a frequent and life threatening event in patients recovering from myocardial infarction. Ventricular tachycardia may be caused by reentry, abnormal automaticity, or triggered activity. Both clinical and experimental studies indicate that the majority of the tachycardias is based on a reentrant mechanism (Wellens et al., 1976, Wellens and Josephson, 1984, Almendral et al., 1986).

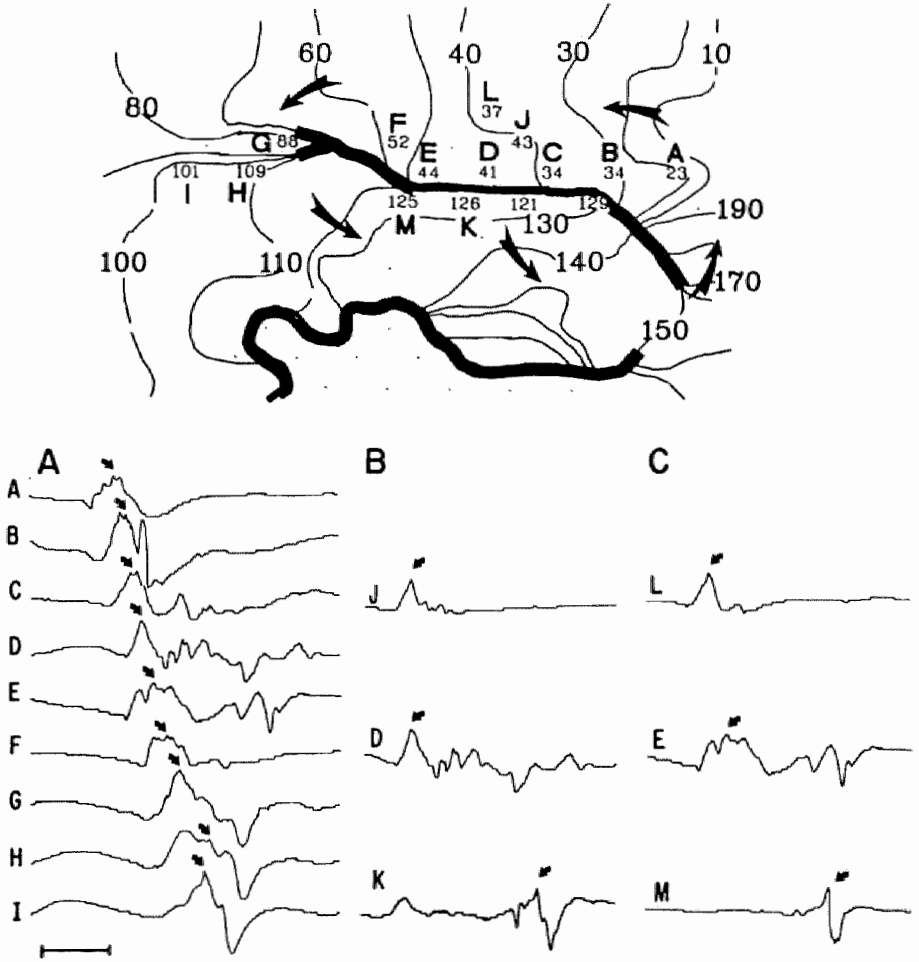
Leading circle reentrant excitation is not a likely mechanism of sustained ventricular tachycardia in the postmyocardial infarction period. Sustained ventricular tachycardia is stable and longlasting due to the presence of an excitable gap (Josephson and Wellens, 1984), whereas during leading circle reentry no excitable gap is present. (Allessie et al, 1984). During the acute phase of a myocardial infarction however, increased inhomogeneity in conduction and refractoriness may cause reentrant arrhythmias based on leading circle reentry. Anatomically determined reentrant excitation involving either scar tissue or myocardial fibers or Purkinje tissue separated by collageneous septa may cause sustained ventricular tachycardias.

In recent years it has been demonstrated by a number of investigators that in canine hearts three to four days after myocardial infarction thin surviving epicardial layers are present. (Wit et al., 1982, 1987, Dillon et al., 1988, El-Sherif et al., 1977a, 1977b, 1981, 1985, Mehra et al.,1983, Gough et al., 1985, Spear et al., 1983a, 1983b)

Figure 5.7. Computer simulation of anisotropic reentry. In this situation one pivoting point (left) was anatomically defined, while the other (right) was determined by the functional properties of the excitable elements. Compared to the anatomically defined circuit (fig 5.5) the length of the line of block had decreased significantly and the cycle length was shortened. Although the excitability of the cells (nr 8) was restored, decremental conduction and conduction block occurred.

These layers, interdigitating with destroyed myocardial fibers and collageneous tissue may play an important role in the pathogenesis of sustained ventricular tachycardias. The resting membrane potential of these surviving cells are shifted to less negative values and the refractory periods may be prolonged, causing unidirectional conduction block and reentry, or may give rise to diastolic depolarization or delayed after depolarizations. (Spear et al., 1983a, 1983b, Wit et al., 1987, Dillon et al., 1988). Spear et al. (1983a, 1983b) demonstrated that within the infarcted region, local continuous activity can be recorded, which can be related to the occurrence of ventricular tachycardia. Local continuous activity caused by slow nonuniform conduction may reflect areas of enhanced tissue anisotropy (Spear et al., 1983a, 1983b, Wit et al., 1987). Dillon et al. (1988) demonstrated that sustained ventricular tachycardias, initiated 3-4 days after myocardial infarction in canine hearts were caused by reentrant excitation within these thin surviving epicardial layers. Although sometimes the central area of conduction block was caused by an anatomical obstacle, in other cases the apparant line of conduction block was determined by the functional properties of the tissue.(figure 5.8A). Although the activation maps showed lines of block orientated parallel to the fiber direction, detailed analysis of the recorded electrograms revealed the presence of slow transverse conduction across the line of conduction block and only a small vortex of circulatingc excitatiornc was present (figure 5.8B). During anisotropic reentry, initiated in our twodimensional model of anisotropy ,the line of conduction block was also orientated parallel to the epicardial fiber direction (chapter 4). However except for a few cases no evidence for slow transverse conduction perpendicular to the line of conduction block was found. This difference is explained by the fact that in healthy myocardium transverse conduction is 3 times faster (20 cm/s) than in the infarcted epicardium (less than 7 cm/s). (Spach et al., 1981, 1986) Further evidence that infarct structure plays an important role in the initiation of sustained ventricular tachycardias was provided by the studies of Kramer et al. (1985) and Pogwizd and Corr

Figure 5.8. Characteristics of electrograms recorded along the line of apperent block. At the top, isochrones from the activation map are shown. The letters indicate recording sites of electrograms shown in panels A, B and C. Below each letter is a number indicating the designated activation time at that site. Panel A shows electrograms recorded at sites A to I, along the line of the block. The arrows indicate the point on the electrogram taken at the moment of activation plotted on the map (see text). Panels B and C show electrograms recorded on either side of the line of block.
(From: Dillon et al., 1988, with permission.)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(1987) who demonstrated that small intramural reentrant circuits may cause sustained ventricular tachycardias in the healing phase after myocardial infarction.

The cycle length of the reentrant tachycardias initiated in canine hearts, ranged from 140 to 240 ms (Dillon et al., 1988), which is somewhat longer than anisotropic reentry in the twodimensional model of anisotropy (105 to 160 ms, mean 130 $\pm$11 ms). This may be explained by ischemic alterations of the cells depressing conduction velocity and lengthening refractory periods.,

In patients with healed infarction and ventricular aneurysms endocavitary mapping studies often reveal extremely broad or fractionated electrograms. These fractionated electrograms are similar to those recorded during ventricular tachycardia near the infarcted region in canine hearts (Josephson et al., 1978, Horowitz et al., 1980, Josephson and Wit, 1984). Although the time course of the anatomic and electrophysiological changes in clinical and experimental studies may be different the pathophysiological basis of arrhythmias is analogous (Fenoglio et al., 1983, Gardner et al., 1985). However in contrast with experimental results, the earliest activation during ventricular tachycardias in patients often originates in the subendocardium of the infarcted region instead of in the epicardium during sustained tachycardias in canine hearts.

The presence of continuous local activity, which reflect sites of altered myocardial structure, however does not implicate that reentrant circuits are located within these regions or that patients are of risk of ventricular tachycardia (Brugada et al., 1985). Direct demonstration that during sustained tachycardia cooling, compression or resection of these regions leads to the termination of the tachycardia is necessary to proove the presence of reentry within these areas (Josephson and Wit, 1984).

The occurrence of sustained ventricular tachycardias diminishes in the course of the healing process. In a recent study however Brugada et al. (1985) demonstrated that in about 40% of the patients periods of ventricular tachycardia could be initiated by programmed electrical stimulation, which indicated that the anatomical substrate for reentrant excitation was still present. The occurrence of "late" tachycardias is related to the presence of ventricular aneurysm and impaired ventricular performance (Jospehson and Wellens, 1984). Despite the fact that in these patients the tachycardia is probably caused by either macro or micro anatomical reentrant circuits enhanced tissue anisotropy of surviving cell layers near the infarcted region, may play an important role in the pathogenesis of these "late" sustained reentrant ventricular tachycardias.

Reentrant excitation can be terminated by locally blocking the conduction of the circulating impulse. The administration of Class I antiarrhythmic drugs may, by depressing the fast sodium inward current result in such a conduction block and termination of reentry.

From the results of Kadish et al. (1986) and the results presented in chapter two, it became clear that class I antiarrhythmic drugs diminish the degree of anisotropy by depressing the longitudinal conduction velocity to a greater extent than the transverse conduction velocity. Another theoretical way class I antiarrhythmic drugs may result in termination of reentrant arrhythmias is preferential conduction block in a transverse directionn. Because transverse conduction in the infarcted region is extremely slow (3-5 cm/s) any further slowing of conduction induced by drug administration may lead to conduction block. Despite the high safety factor for conduction, due to a high initial Vmax of the action potentials, conduction may be critical during these low velocities (Van Capelle, 1983, Rudy et al, 1987).

Apart from changes in active membrane properties also the passive membrane properties play a key role in conduction and conduction disturbances in the anisotropic myocardium. Although the structure of infarcted regions will be different and more complex, the model of anisotropy presented in this study may be useful to study the mechanisms of sustained ventricular tachycardia to evaluate the effects of antiarrhythmic drugs.

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SUMMARY

The aim of the study presented in this thesis was to investigate the role of tissue anisotropy on conduction and conduction disturbances in the Langendorff perfused rabbit left ventricle.

In chapter one, a short introduction concerning impulse conduction and conduction disturbances is given. The possible role of tissue anisotropy in the pathogenesis of reentrant arrhythmias is discussed and the various electrophysiological parameters involved in the initiation of cardiac arrhythmias are evaluated.

In chapter two the effects of heart rate, premature beats, potassium, temperature, epinephrine and lidocaine on conduction velocity, refractory period, and the wavelength of the cardiac impulse are described. Due to the anisotropic morphology of the left ventricle, conduction velocity of the cardiac impulse depends on the direction of impulse propagation. Parallel to the fiber direction, impulse conduction is about three times faster than perpendicular to the fiber direction. Consequently the wavelength of refractoriness depends on the direction of impulse propagation. The degree of anisotropy (the ratio between the longitudinal and tranverse conduction velocity) may vary from less than 1.5 (during high extracellular potassium) to more than 3.4 (during cooling). However in the intact rabbit ventricle it is difficult to correlate the wavelength with the risk of the initiation of reentrant arrhythmias. This is due to the fact, that the three dimensional architecture of the ventricles effectively counteracts the arrhythmogenic effects of slow transverse conduction.

To study the effects of tissue anisotropy on conduction of the cardiac impulse a new experimental model of anisotropy was developed to create a thin epicardial layer of normal ventricular myocardium (chapter three). A Langendorff perfused rabbit heart was immersed in a warm tissue bath and a cryoprobe inserted in the left ventricular cavity. By endocardial freezing the total interventricular septum and 4/5 th of the free wall of the left ventricle were destructed. Only a 1 mm thick epicardial layer, protected by the warm perfusion fluid in the surrounding tissue bath, survived. This surviving layer of epicardium showed the same electrophysiologic properties as before freezing, both refractory periods and conduction velocity being the same as during control. Epicardial conduction was mapped with a 192 fold mapping electrode (Spatial resolution 1 mm). It was found that in the intact ventricle slow transverse conduction

was interrupted within 3-4 mm from the site of stimulation by epicardial breakthrough from a faster propagating intramural wavefront. However in the thin epicardial layer slow transverse conduction could proceed undisturbed resulting in an extension of the area of effective anisotropy. Longitudinal and transverse conduction were studied during high extracellular potassium, induction of premature beats and rapid pacing. Under all these circumstances local conduction block occurred more readily in a transverse direction than in a longitudinal direction. However, although arcs of transverse conduction block occurred more frequently, they rarely led to intramyocardial reentry because the tissue distal to the line of conduction block was activated with little delay by fast longitudinal propagation. In contrast, the area distal to a longitudinal block was activated with more delay by a slow transverse wavefront, creating a higher chance of reentry of the fibers proximal to the line of block. Induction of intramyocardial reentry by (multiple) premature beats or rapid pacing resulted in episodes of sustained monomorphic ventricular tachycardia. This led to the conclusion that the removal of the subepicardium enlarges the effective area of anisotropy in the overlying epicardium facilitating the induction and perpetuation of reentrant ventricular tachycardia.

In chapter four, the characteristics and mechanisms of sustained ventricular tachycardias initiated in the two dimensional model of anisotropy are presented. The tachycardias could be initiated by either incremental pacing or programmed electrical stimulation, aplying upto three shortly coupled premature beats. From the reconstructed epicardial activation maps it became clear that the tachycardias were caused by *circusmovement excitation*, during which the impulse was circulating around a long, functionally determined line of conduction block which was orientated parallel to the epicardial fiber direction. Despite the fact that the line of block was functionally determined, an excitable gap was present. Due to the presence of an excitable gap tachycardias were stable and longlasting.

In chapter five the effects of enhanced tissue anisotropy on conduction disturbances and reentrant ventricular tachycardias is discussed. The characteristics of "anisotropic" reentry, as a new type of circusmovement, are summarized and compared with the properties of anatomically determined reentrant excitation and leading circle reentry. The possible mechanisms of the creation of an excitable gap during "anisotropic" reentry are analysed and the preliminary results of a computer simulation study are presented. Furthermore, the possible clinical significance of "anisotropic" reentry

as a mechanism of sustained ventricular tachycardia in the healing phase after a myocardial infarction in patients is discussed.

We conclude that:

1. The three dimensional geometry of the ventricles prevents the initiation of reentrant arrhythmias in the intact heart.
2. Enhanced tissue anisotropy facilitates the induction of reentrant ventricular arrhythmias.
3. "Anisotropic" reentry may be a likely mechanism of sustained ventricular tachycardia in the healing phase after a myocardial infarction.
4. The two dimensional model of anisotropy, presented in this thesis, is a useful tool to study the mechanisms of ventricular tachycardias and to evaluate the effects of antiarrhythmic drugs.

SAMENVATTING

Afwijkingen van het normale slagritme van het hart kunnen verschillende consequenties hebben, varierend van een slechts minimaal verminderde pompfunctie tijdens extrasystoles tot een volledig falen van de pompfunctie tijdens kamerfibrilleren, leidend tot acute hartdood.

In het gezonde hart, wordt ongeveer 70 maal per minuut een electrische impuls gevormd door de cellen in de sinusknoop. Deze impuls verspreidt zich snel over de beide boezems en bereikt na een fractie van een seconde de atrioventriculaire knoop gelegen op de scheiding tussen boezems en ventrikels. Na een geleidingsvertraging in deze structuur versnelt de impuls weer en verspreidt zich via een speciaal geleidingssysteem (het Purkinje systeem) snel over beide kamers, waarna de kamercellen worden bereikt. Na electrische prikkeling van de kamercellen zullen deze min of meer synchroon samen trekken. De volgorde van activatie en contractie van de kamercellen is van belang voor een normale pompfunctie. Er zijn echter verschillende ziektebeelden waarbij de normale impulsgeleiding gestoord is en de pompfunctie van het hart is verminderd. Met name na een myocardinfarct wanneer een deel van de hartspier is afgestorven kan dit leiden tot ernstige afwijkingen van het normale hartritme.

Hartritmestoornissen kunnen worden veroorzaakt door drie verschillende mechanismen; 1. Aritmieën veroorzaakt door abnormale impulsvorming, 2. Aritmieën veroorzaakt door abnormale impulsgeleiding, 3. Aritmieën veroorzaakt door een combinatie van abnormale impulsgeleiding en impulsformatie.

In dit proefschrift worden de eigenschappen bestudeerd van aritmieën die veroorzaakt worden door abnormale impulsgeleiding.

Abnormale impulsgeleiding in het kamermyocard kan, indien geleidingsblok optreedt in een richting en de impuls traag geleid wordt in de andere richting, leiden tot ritmestoornissen, waarbij de impuls "gevangen" raakt in een cirkelvormig activatiepad. Alhoewel locaal geleidingsblok en trage geleiding kunnen optreden als gevolg van locale verschillen in prikkelbaarheid van de cellen, blijkt uit recente studies, dat deze geleidingsstoornissen ook kunnen optreden als gevolg van verschillen in geleidingseigenschappen afhankelijk van de richting van impulsgeleiding. In tegenstelling tot in de boezems, zijn de cellen in de ventrikels min of meer parallel aan elkaar gerangschikt. De cellen zijn aan elkaar gekoppeld door middel van een aantal "weer-

stands"- bruggen. Omdat hartspiercellen langer zijn dan breed, worden er veel meer "weerstands"- bruggen in de dwarse richting gevonden. Als gevolg hiervan, is de weerstand dwars op de vezelrichting een factor 9-11 hoger dan de weerstand parallel aan de vezelrichting. Omdat de geleidingssnelheid van de impuls omgekeerd evenredig is aan de wortel van de weerstand, is de geleidingssnelheid parallel aan de vezelrichting ongeveer 3 maal zo snel als de geleidingssnelheid in transversale richting (Anisotropie). Geleidingsnelheid, refractaire periode, en de golflengte van de impuls zijn belangrijke electrofysiologische parameters, die de gevoeligheid van het myocard voor het optreden van hartritmestoornissen gebaseerd op cirkel geleiding bepalen.

In hoofdstuk twee wordt het effect van weefselanisotropie op deze parameters en de effecten van snel drijven, extrasystoles, hoog kalium, hypothermie, adrenaline en lidocaine beschreven. Het was echter niet mogelijk de kans op het optreden van ritmestoornissen te correleren met bovengenoemde parameters omdat het in het intacte geperfundeerde konijnenhart zeer moeilijk was om hartritmestoornissen op te wekken. Alleen na langdurige, zeer snelle electrische stimulatie onstond kamerfibrilleren. Daarnaast werden betrouwbare epicardiale metingen gecompliceerd door de driedimensionale geometrie van de linker kamer en bleek dat de aritmogene effecten van trage transverale impulsgeleiding worden gemaskeerd door snelle impulsgeleiding via intramurale- en endocardiale spierlagen.

Een nieuw biologisch model, ontwikkeld om deze problemen op te lossen, wordt gepresenteerd in hoofdstuk drie. Het, volgens de Langendorff techniek geperfundeerde, konijnenhart werd in een verwarmd weefselbakje geplaatst. Door middel van een in de linkerkamer geplaatste cryoprobe werd het totale intraventriculaire septum en 4/5-de deel van de binnenzijde van de linker kamer doodgevroren. Slechts een 1 mm dikke epicardiale laag overleefde deze procedure. Na vriezen waren de electrofysiologische eigenschappen van dit overlevende laagje onveranderd: de refractaire periode en de geleidingssnelheid waren niet verschillend van de waarden gemeten tijdens controle omstandigheden in het intacte hart. De epicardiale geleiding werd gemeten met een "mapping" electrode bestaande uit 192 verschillende electrodes, met een spatiele resolutie van 1 mm. In de intacte ventrikel werd langzame transversale geleiding op een afstand van 3-4 mm van de epicardiale prikkel plaats onderbroken door snelle intramurale- en endocardiale impulsgeleiding. In de dunne overlevende epicardiale schil echter was de effectieve anisotropie toegenomen omdat langzame transversale geleiding niet meer werd onderbroken door een snelle impulsgeleiding in

diepere lagen. In dit model werd de invloed van hoge concentraties extracellulair kalium, snelle electrische stimulatie en extra-systoles op geleiding bestudeerd. Het bleek dat onder alle omstandigheden van verminderde effectiviteit van de impuls geleidingsblok eerst optrad tijdens trage transversale impulsgeleiding. Abnormale impulsgeleiding in deze gebieden kon aanleiding geven tot ritmestoornissen gebaseerd op cirkelgeleiding van de electrische impuls.

De eigenschappen van deze cirkeltachycardieën, opgewekt in het tweedimensionale model van de linker kamer, worden beschreven in hoofdstuk vier.

In hoofdstuk vijf worden de eigenschappen van "anisotrope" reentry vergeleken met cirkeltachycardieën waarbij de impuls draait rondom een anatomisch obstakel of rondom een functioneel blokgebied in isotroop weefsel. Voorts worden de cirkeltachycardieën, opgewekt in dit experimentele model, vergeleken met kamertachycardieën die optreden in de genezingsfase van een myocardinfarct.

Uit de experimentele resultaten beschreven in dit proefschrift blijkt dat:

1. De driedimensionale structuur van de hartkamers een efficiente bescherming biedt tegen het ontstaan van cirkeltachycardieën in het gezonde myocard.
2. De toename van weefselanisotropie na een myocardinfarct een belangrijke rol kan spelen bij het optreden van kamerritmestoornissen.
3. Anisotrope reentry een belangrijk mechanisme is voor het ontstaan van kamertachycardieën in de herstelfase van een myocardinfarct.
4. Het door ons ontwikkelde, tweedimensionale, model van de linker kamer een nuttig hulpmiddel kan zijn bij het onderzoek naar geleidingsstoornissen en mechanismen van cirkeltachycardieën in het kamermyocard.

NAWOORD

Indien je na een treinreis, van voor Nederlandse begrippen ongekend lange duur, eindelijk in Maastricht aankomt en je na enig zoeken het biomedisch centrum binnenkomt, om je vervolgens voor ongeveer vier jaar in een avontuur met onbekende afloop te storten, weet je niet wat er vast zit aan "Een paar jaar onderzoek lijkt me wel leuk". Ik kijk echter met veel plezier op deze periode terug en ben prof. Allessie en prof. Bonke dan ook dankbaar dat zij mij deze kans gegeven hebben.

De vrijheid binnen het werk en de mogelijkheid om "leuke" dingen te doen, met de zekerheid dat je altijd op Maurits en Vic terug kon vallen was iets geweldigs. De wijze waarop Maurits het onderzoek begeleidde was voor sommigen misschien niet altijd even begrijpelijk, maar als je daar eenmaal aan gewend was geraakt toch geweldig stimulerend. Beste Maurits, hartelijk dank daarvoor. De discussies onder het genot van een Javaanse jongen en een grote gele, zal ik niet licht vergeten en zeker missen. Dat Vic na enige tijd rector werd, mocht hem er niet van weerhouden ons werk critisch te volgen. Uit de wijze waarop je het manuscript beoordeeld hebt, blijkt dat je betrokkenheid bij de electrofysiologie niet geleden heeft onder de lusten en lasten van het besturen.

Naast Maurits en Vic waren er nog een aantal mensen die van essentieel belang zijn geweest voor het tot stand komen van dit proefschrift.

Prof. Reneman, beste Rob, de manier waarop jij al gedurende vele jaren de vakgroep fysiologie leidt, kan alleen maar tot voorbeeld strekken voor iedereen en heeft geleid tot een min of meer hechte groep mensen waarmee het goed samenwerken is.

Het feit dat mijn cardiologie opleider prof. Bruschke het mogelijk maakte om zonder zorgen voor de toekomst rustig aan dit onderzoek te kunnen werken speelde zeker een belangrijke rol, daarvoor mijn hartelijke dank.

De recente samenwerking met Frans van Capelle, waarvan de eerste resultaten in hoofdstuk vijf van dit proefschrift zijn beschreven, heeft geleid tot een nieuwe lijn van onderzoek die veelbelovend lijkt te zijn. Het was dan ook een groot genoegen dat je het manuscript wilde beoordelen.

De samenwerking met Prof. Wit op het gebied van de ventriculaire tachycardieën was stimulerend en, beste Andy, het is dan ook een eer voor mij dat je aanwezig kunt zijn op mijn promotie.

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Het secretariaat, ook in deze tijd nog steeds onmisbaar bij het tijdig klaar krijgen van manuscripten en verantwoordelijk voor de goede gang van zaken binnen de vakgroep. Met name Luclenne de Boer vervulde daarin een grote rol samen met Karin van Brussel, Jos Heemskerk en Emmy van Roosmalen.
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Curriculum Vitae

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